Report of the NIAID Task Force on Immunology

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Contents

Foreword

The past two decades of intense and highly productive research on the immune system have resulted in a wealth of new information and extraordinary growth in conceptual understanding. These accomplishments now provide realistic opportunities for major advances in the diagnosis, prevention, and treatment of a broad range of human diseases. The study of immunology has come of age. Despite the considerable gaps in knowledge that still exist, an understanding of the complexities of immune activation and regulation within the physiological framework of the whole organism is now emerging. As we enter the next century, these advances provide a solid foundation for translating basic research into clinical applications.

The challenges for future immunological research are many. The movement toward a global economy highlights the enormous differences among nations in the prevalence of infectious diseases and accessibility to health care; the increased risk of global epidemics is very clear. The examples provided by human immunodeficiency virus (HIV)/AIDS, as well as newly emerging and reemerging infectious diseases, increased antimicrobial drug resistance, and widespread foodborne infections in recent years, demonstrate the need for expanded efforts to develop new and affordable vaccines for a broad range of pathogenic microorganisms. The growing recognition of associations between certain types of cancer and chronic infections also serves to emphasize the enormous potential impact of vaccines on human health. We are just beginning to understand the immune parameters of tumor growth and neurological and reproductive diseases, and dedicated research will open these areas for future clinical applications.

In the 8 years since publication of the 1990 Report of the NIAID Task Force on Immunology and Allergy, technological advances in gene targeting have allowed the creation and analysis of many new animal models of immunodeficiency, autoimmunity, and transplant rejection, and have revolutionized the ability to accurately map and sequence entire genomes to identify variant genes that contribute to disease. Advances in structural biology have increased our understanding of immunologically important molecular interactions. The recent three-dimensional descriptions of TCR-MHC-antigen and CD4-HIVgp120 complexes at the atomic level are good examples of results that provide essential information on functionally relevant protein domains and novel targets for immunomodulation and drug discovery. Remarkable progress over the past few years in defining the molecular, cellular, and systemic parameters of antigen processing and presentation by MHC molecules is providing important information for new vaccine development. A number of novel cytokines, chemokines, and their receptors have been identified; and new insights into the mechanisms of receptor-mediated signaling and the control of

gene transcription have led to a greater understanding of cellular behaviors and the systemic regulation of immunity. Continued work in these areas, enriched by the enormous progress expected in related fields of biomedical research and enlightened by a strengthened emphasis on the human immune system, should provide a comprehensive picture of immune regulation in human health and disease and enable the development of new immune-based therapies.

Another major advance in the past decade was the identification of mechanisms by which antigen-specific immune tolerance can be induced to prevent pathologic immune responses. Many human disorders result from inappropriate or uncontrolled immune reactions that cause damage to the individual. For example, more than 15 percent of North Americans, Europeans, and Japanese suffer from allergic diseases, and autoimmune diseases affect nearly 5 percent of the adult population of these countries. Based on the more comprehensive knowledge of immune tolerance that is now available, clinical applications to immune-mediated disorders such as allergy, asthma, autoimmune disease, and transplant rejection are being developed, and early clinical trials are already in progress.

In light of the impressive recent progress in basic research and clear opportunities for a broad range of future clinical applications, it is appropriate on this 50th anniversary of NIAID to examine the most promising research directions in a number of areas relevant to immunology. Thus, a task force was convened to define the present state of immunological research and to develop a thoughtful and comprehensive set of recommendations for future research to aid in planning NIAID strategies aimed at improving human health. The cochairs of each chapter of this Task Force Report developed the scientific topics and enlisted more than 140 expert scientists to concisely review recent advances and define promising directions for future research. We are indebted to these distinguished cochairs and contributors who generously volunteered their time, expertise, and insights in this collaborative effort. I also would like to acknowledge the outstanding work of Dr. Alfred Nisonoff, who served as executive secretary for the Task Force, and to thank the staff of the Division of Allergy, Immunology and Transplantation for their help in compiling the report. I hope that this summary of opportunities in basic and translational research proves to be informative and useful as a planning tool for its readers. It will serve as a guide for NIAID in planning future initiatives to advance progress in vaccine development and in the diagnosis, prevention, and treatment of allergic and immunological diseases.

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Executive Summary

Nature of the Immune System

It has become increasingly clear over the past decade that the antibody and T cell responses of the adaptive immune system are influenced by the cells and soluble products of the innate immune system. Macrophages, neutrophils, and other cells that mediate innate immunity form a first line of defense against pathogen invasion, helping to limit infection until antigen-specific B and T lymphocyte responses are induced. One of the most exciting advances of recent years was the realization that innate responses serve to regulate the onset, duration, magnitude, and character of antibody- and cell-mediated adaptive responses. The demonstration of these important roles for innate immunity and a growing appreciation of the underlying mechanisms at the molecular level provide opportunities for new vaccine strategies. Thus, innovative approaches will target cells of the innate immune system as well as T and B lymphocytes to generate potent immunity of the appropriate type to protect against acute infection and stimulate clearance during chronic infection.

Many of the cells involved in both the innate and adaptive immune systems circulate throughout the body. Lymphocytes also home to specialized areas of the spleen and lymph nodes where they multiply and become potent effector cells upon encounter with pathogenic antigens. They then migrate to areas of infection. Increasingly sophisticated experimental approaches are being used to identify the molecular guideposts and signals that direct traffic for the appropriate localization of immune cells, and these trafficking signals are now being studied as targets for therapeutic intervention.

Considerable progress has been made in defining structures and functions of molecules important to immune responses. Molecular mechanisms by which specific antigen receptors are assembled and expressed on T and B cell surfaces, and lymphocyte selection mechanisms that ensure an antigen-reactive repertoire ready to fight disease while maintaining self-tolerance, have been identified. Although questions remain, enormous progress has been made in identifying and characterizing the intracellular molecules that control the effector functions elicited by antigen binding. These effector functions are critical for combating infection, and also are responsible for allergic reactions, autoimmune attacks, and transplant rejection. Notable advances in characterizing these mechanisms include a more thorough understanding of the secreted, cytolytic mediators that kill tumor cells or infected cells, as well as the cell surface and intracellular proteins involved in controlling cell death. The orchestration of particular types of immune responses by different T cell subsets and cytokines has also been clarified. These accomplishments provide a foundation for future work that will target individual molecular components to improve vaccine development and therapies for immune-mediated diseases.

It has been known for some time that immune responses in the skin and epithelial surfaces of the body, such as lung and intestine, may differ from systemic immune responses in important ways. Because these surfaces are the most common sites of entry for allergens and infectious pathogens, it is important to understand how immunity is controlled at these sites. Although considerable progress was made in recent years, renewed emphasis on this area of research is warranted, and the genetic, biochemical, and cellular tools are now available for more definitive studies in the next decade of research. The rewards of such efforts will include novel vaccine strategies as well as immunomodulatory regimens to control allergic responses.

Development of the Immune System

A comprehensive understanding of the maturation of lymphocytes and other cells involved in immune function is required to adequately address serious clinical problems such as primary immunodeficiency diseases and immune reconstitution following treatment of HIV or cancer. Among the many scientific advances of the past decade are the isolation and characterization of hematopoietic stem cells that differentiate into virtually all of the mature cell types important in immune responses. This maturation process occurs throughout life because the mature cells have relatively short lifetimes and they must be renewed continuously. This fascinating area has attracted considerable research attention in recent years. Gene knockout mice and molecular biology techniques are being used to identify and manipulate key components of differentiation in experimental systems. The complex series of molecular events that control the development of different cell types from a single precursor are being defined. Progress has also been made in understanding the regulation and terminal differentiation of mature lymphocytes, and future work will define precise mechanisms that can be manipulated to enhance, inhibit, or alter the type of immune response that can be generated.

Developmental defects in the negative selection of lymphocytes contribute to autoimmune disease. If normal deletion of self-reactive T or B cells does not occur, the potential for tissue destruction by autoaggressive lymphocytes may be unleashed. Over the past 15 years, intense research on the mechanisms of self-tolerance has led to a greater understanding of tolerance regulation. This work provides exciting new opportunities for clinical applications in transplant rejection, allergy, and asthma, as well as the control of autoimmune disease. Research in the next decade will emphasize *in vivo* animal studies and clinical trials to test tolerance-inducing protocols and translate basic research findings into therapies for a broad range of immune-mediated disorders.

Primary Immunodeficiency Diseases

Inherited defects in immune function are responsible for a variety of human diseases that range from mild to fatal. In rare cases, neither B nor T cells develop, resulting in the "boy in the bubble" syndrome in which affected individuals are extremely susceptible to infection and die within a year of birth if not reconstituted with normal bone marrow. Although

many of the gene mutations that cause primary immunodeficiency disease were identified recently, many more have not yet been defined. This task will require the cooperative efforts of many scientists using molecular biology approaches, new information from the human genome project, and extensive family studies. Once the genes are identified, it will be important to define the physiological roles of the proteins they encode; furthermore, animal models in which the relevant genes have been knocked out will constitute valuable resources.

Immunoregulation

Protective immunity requires powerful cellular responses that must be tightly regulated in order to minimize damage to the host while allowing the destruction of pathogens. Different types of immune effector functions are needed to combat different pathogens. There is good evidence that certain immune effector molecules can inhibit the generation or activity of other effector molecules needed to fight infections by different pathogens. For example, the cytokine interferon-gamma prevents the production of interleukin (IL)-4 and IL-10, and IL-10 inhibits IL-12 production. Thus, in order to restore homeostasis, efficient immune downregulation occurs once a foreign invader is destroyed.

Because lymphocytes usually proliferate extensively after recognition of their antigens and become effector cells, recovery from the immune response also involves the death of many of the progeny cells once the antigen is cleared. Defects in the process of cell death can cause serious problems due to uncontrolled lymphoproliferation and the accumulation of too many lymphocytes in the body. The identification and characterization of many of the proteins that regulate cell death were achieved in recent years. The immune response also generates memory lymphocytes. Memory cells do not die quickly but persist in a more sensitive state to provide rapid protection against subsequent encounters with the same antigen. Although the creation of memory lymphocytes is an important goal of vaccine development, little is known about the factors that control the generation and maintenance of these cells over long periods of time.

Opportunities to expand our current knowledge of immunoregulatory mechanisms are plentiful. For example, T and B lymphocyte subsets that perform different biological functions have been identified. Current technologies can be used to characterize more fully the cell surface receptors, intracellular signaling pathways, and proteins that control gene transcription and mediate effector functions of these subsets. Numerous soluble mediators of immunity, such as cytokines, chemokines, and cytotoxic enzymes, have already been identified, and it is likely that more will be discovered. As yet, little is known about the immunoregulatory functions of factors such as glucocorticoids, prostaglandins, sex hormones, and neurotransmitters, and research in these areas should be highly productive. Work is also needed on the regulation of immunity at different sites in the body, such as the mucosal surfaces and the brain. Finally, although considerable advances in understanding immune tolerance resulted from

recent studies, continued work is needed to provide information important for the rational development of tolerogenic regimens to control allergy, autoimmunity, and transplant rejection. Advances in all these areas will involve a shift in focus from animal models to human studies.

Inflammation and Host Defense

Inflammation is the complex process by which white blood cells, soluble proteins, and extracellular matrix components combine to localize and kill an invading pathogen. In some cases, the pathogen may not be completely eliminated, resulting in chronic inflammation that can itself damage normal tissues. Furthermore, inappropriately triggered inflammatory responses can contribute to the pathogenesis of a variety of diseases, such as diabetes, rheumatoid arthritis, asthma, atherosclerosis, and Alzheimer's disease. The goal of current and future research is to reverse chronic inflammation and regulate inflammatory cells such that efficient antimicrobial defense can occur with minimal damage to host tissues.

Major advances in the past decade include the application of molecular biology techniques to identify previously unknown soluble factors, such as cytokines and chemokines, that regulate white blood cell migration and antimicrobial activity, and adhesion molecules that control leukocyte trafficking. A much more sophisticated understanding of the signaling mechanisms that turn on the potent effector functions of inflammatory cells was also achieved. Continued work in these areas will facilitate the development of anti-inflammatory drugs to control this important mechanism of host defense, with applications to a broad range of human diseases. A greater understanding of the molecular events responsible for the normal, spontaneous resolution of inflammation will provide much needed information for such translational research.

Immediate Hypersensitivity and Allergy

Some of the most common human disorders result from allergic responses, which are detrimental immune reactions to inhaled, ingested, or injected antigens. The high incidence of allergic diseases has major social and economic implications. Many allergic responses are debilitating, and some, such as systemic anaphylaxis, can be life threatening. Furthermore, continuous exposure to an allergen can cause chronic inflammation, as seen in atopic dermatitis, allergic rhinitis, and some forms of asthma. Recent advances in understanding the induction and control of allergy include identification of the primary structures of many allergens and their particular molecular regions, or epitopes, that activate pathogenic T and B cells. Many of the harmful effects of allergens are mediated by the antibody type called IgE. Much is now known about the mechanisms by which IgE:allergen complexes trigger the release of histamine and other soluble mediators from circulating basophils and mast cells in tissues. Non-IgE-dependent mechanisms of allergy also exist, and further work is needed to clarify the complex physiology of allergic responses.

One major research focus during the past decade has been the role of different T

cell subsets and their secreted cytokines in either inducing or inhibiting allergic reactions, and this area holds promise for therapeutic intervention. Another category of allergic mediators, the eicosanoid and prostanoid lipids, may also serve as therapeutic targets for the control of allergy. A role for some of these compounds in asthma is now well established, and newly developed drugs that target these molecules are proving effective. Thus, expanded studies on this class of soluble mediators are likely to be very productive. There is considerable evidence pointing to a genetic predisposition in allergic individuals, and it is clear that many different genes are involved. Although sorting out the genetic patterns that distinguish allergic from nonallergic individuals is a complex process that will continue for many years, substantial progress has already been made in identifying candidate gene loci in defined human populations.

Asthma

The prevalence of asthma in the United States and other developed countries has increased considerably in recent years for reasons that are not well understood. Responses to a variety of inhaled substances and respiratory infections exacerbate chronic asthma. The mechanisms responsible for the underlying inflammatory disease and acute asthmatic episodes are being studied intensely in both humans and animal models. Bronchial hyperreactivity, altered smooth muscle function, epithelial immunopathology, and airway fibrosis and obstruction characterize the asthmatic state. The precise role of the mucosal immune system is not yet known, but recent advances have indicated that infiltrating T cells promote inflammation, and a variety of cytokines, chemokines, and cell adhesion molecules work in concert to recruit other inflammatory cells into the lung.

Immunosuppressive corticosteroid treatment is effective in reducing asthma symptoms, and research has focused on soluble immune mediators that might serve as more specific targets for future immunotherapy regimens. Such mediators include IL-4, which upregulates adhesion molecules and IgE production; eotaxin and IL-5, which recruit and stimulate eosinophils; IL-10, which stimulates macrophages in the alveoli; and leukotrienes, which promote submucosal edema and bronchial constriction. Studies to map the genetic loci that influence susceptibility to asthma have already begun, and the results thus far confirm the multigenic nature of this disease and the heterogeneity of asthma phenotypes. Continued large-scale cooperative efforts among scientists are needed to complete such genetic studies. Areas of emphasis include identification of the specific genes involved, elucidation of their functions and relative importance in the disease process, and their association with different types of asthma. Extensive epidemiological work to determine the natural history of asthma and to characterize the particular effects of individual asthma-inducing stimuli is also needed, as are continued basic studies on the immunoregulation of the asthmatic process. Future advances in prevention and treatment will depend on highly coordinated, multidisciplinary scientific efforts to understand the complex genetics and biology of this debilitating disease.

Autoimmunity

Immune dysregulation can result in destructive immune responses to self tissues and the progressive loss of neural, renal, pancreatic, or other vital organ functions. Collectively, autoimmune diseases afflict nearly 5 percent of the American population. Examples include multiple sclerosis, systemic lupus erythematosus, type 1 diabetes, and rheumatoid arthritis. These are chronic, severe diseases that cause a tremendous burden of human suffering and economic loss. As with many other immune-mediated disorders, there is a genetic component, and multiple susceptibility genes are involved that may differ among ethnic populations. Progress in mapping these genes is already evident, and extensive work is required to identify the relevant patterns of gene expression and the functions of the gene products in disease pathogenesis.

Although it is clear that autoimmunity results from a breakdown in self tolerance, the initiating events are not yet known. Tissue damage may play a role, by increasing self antigen concentrations or by making self antigens more accessible to the immune system. Infectious organisms may also play a role, either by disrupting immune homeostasis and activating normally quiescent, low-affinity, self-reactive lymphocytes, or by stimulating pathogen-reactive lymphocytes that can also recognize self antigens and thereby trigger crossreactive responses to self tissues. Recent advances in understanding the pathogenesis of autoimmune diseases include characterization of different T cell subsets and their cytokine products that either mediate autoimmune destruction or protect against it. These findings, together with a more complete understanding of the molecular mechanisms by which self tolerance is maintained in healthy individuals, provide a strong foundation for the development of immune-based, antigen-specific therapeutic protocols to reverse autoimmune attack, restore normal self tolerance, and perhaps prevent disease initiation in susceptible individuals.

Transplantation

The current success of organ transplantation resulted from years of research that developed surgical, medical, and immunosuppressive techniques to allow the replacement of diseased vital organs with healthy ones. Most organ recipients differ from their donors at multiple genetic loci, and the immune system is exquisitely sensitive to the foreign antigens encoded by these genes. Thus, it was imperative to find methods to prevent immune attack on transplanted tissues. One major advance during the 1980s was the introduction of the drug cyclosporin A, which suppresses the entire immune system. In conjunction with other therapies, cyclosporin A made it possible to achieve impressive rates of graft and patient survival.

These life-saving developments have an enormous impact on health care, but they come with a price. General immunosuppression such as that caused by cyclosporin A requires life-long drug therapy that makes the recipient highly susceptible to infections, tumors, and drug-related complications. Therefore, recent work has focused on methods to induce specific immune tolerance by eliminating responses from lymphocytes that recognize the foreign antigens

expressed on the grafted tissues. This approach would leave the rest of the immune system intact and ready to combat infections and incipient cancer cells. On the basis of remarkable recent progress in understanding antigen-specific tolerance mechanisms, scientists are now conducting nonhuman primate preclinical trials of tolerance-inducing regimens that show promising results for translation to human transplant therapies. Furthermore, because these potential therapies target fundamental processes of immune activation, they may be applicable to other immune-mediated diseases such as allergy, asthma, and autoimmune disease.

Many barriers to the successful transplantation of certain organs and tissues remain. For example, the functions of transplanted organs often begin to fail after several years, due to processes of chronic rejection that are not yet well understood. Some cellular transplants, such as the pancreatic islets needed for insulin production, have not been as successful as heart, liver, or kidney transplants. More work is needed in these areas to define the immunological approaches that might ensure good engraftment and functional regulation. Finally, the current success of transplantation has already made it clear that donor organ shortage will become an increasingly difficult problem to address. Thus, animal donors are being considered for future transplantation of a variety of organs and tissues. This area of research, called xenotransplantation, has seen major progress over the past decade, with breakthroughs in suppressing immune reactions that are unique to xenotransplantation. Much work still lies ahead and questions concerning possible transmission of animal viruses to humans have not yet been resolved. However, scientists are optimistic that animal donors can be used in future protocols to replace the very limited sources of normal human tissues.

Tumor Immunology

It is now known that T cell-mediated immunity can effectively eliminate B cell lymphomas associated with the Epstein-Barr virus. A number of other infectious agents have been linked with cancer development, such as the human papillomavirus and cervical cancer, human herpes virus 8 and Kaposi sarcoma, *Helicobacter pylori* and stomach cancer, hepatitis B virus and liver cancer, and human T cell lymphotropic virus-1 and T cell leukemia. The potential for immune elimination of such cancer cells has not yet been established, but prevention of infection is likely to reduce cancer incidence. Other types of cancer, not associated with infectious agents, express normal self proteins in abnormal ways, or tumor-specific proteins encoded by mutated genes in the cancer cells. Such proteins might stimulate immune activation and tumor destruction under appropriate conditions.

Recent preclinical advances include the identification of many tumor antigens and their use in experimental vaccines to induce potent antitumor immune responses. The delay and actual reversal of tumor growth have been demonstrated in animal models. A number of research groups were successful in boosting tumor immunity by incorporating immune-enhancing cytokines into engineered vaccines. A second approach involves

loading antigen-presenting cells with tumor-associated antigens *in vitro* to generate antitumor immune responses following injection of the cells *in vivo*. Antigen-specific antibody treatment has also shown promise for the elimination of certain tumors. Many of these immunologically based approaches have been very effective in animal cancer models when used soon after the introduction of tumor cells, but it is not yet known whether they can reverse the growth of large, well-established tumors. Nonetheless, they may prove useful in the elimination of residual cancer cells following conventional surgery, radiation, or chemotherapy to reduce tumor mass.

Scientists are beginning to define the mechanisms by which antigen-specific lymphocytes can destroy tumor cells, as well as the varied mechanisms by which cancer cells often evade the immune system. Inflammatory responses can either enhance or inhibit malignant cell growth depending on the particular model system studied, and more work is needed to understand the roles of inflammatory cells and cytokines in modulating tumor development. Remarkable advances in defining the basic biology of both tumor cells and immune cells have set the stage for future development of immunostimulatory approaches to eliminating malignancies.

Reproductive Immunology

Despite the potential for fetal rejection by the maternal immune system, reproduction is quite successful in genetically incompatible outbred populations. In most cases, local immune tolerance to the paternal antigens is established spontaneously to allow fertilization, implantation, pregnancy, and birth while maintaining adequate systemic immune competence in the mother. The reproductive process thus represents a form of naturally occurring immune tolerance to foreign antigens. Nonetheless, in some cases immune responses do cause infertility or spontaneous abortion in humans and other animals. The study of these abnormalities may provide information critical for understanding reproductive health.

Research to date has focused on the immunological parameters of fertility and pregnancy in order to identify the cell types, cytokines, and molecular interactions that prevent rejection and promote fertilization and fetal development. Much remains to be learned, and future research can use a multitude of technologies and a broad foundation of immunological knowledge to solve the fascinating and complex problems of reproductive tolerance at the molecular level. There are many potential benefits of advances in this area. New approaches to contraception, infertility, and the prevention of spontaneous abortion can be readily envisioned. In addition, the tolerance mechanisms identified may be applicable to other situations, such as organ transplantation, allergy, and autoimmunity. A greater understanding of the mucosal immune system will surely result, with applications for new vaccine development. This specialized area of research offers a multitude of opportunities for improving human health.

Neuroimmunology

Functionally important interactions between the nervous system and the immune system have been observed for many years, but scientists are just begin-

ning to identify and characterize the molecular mechanisms responsible for neural influences on immunity and immune involvement in cognitive and behavioral activities. This is a complex area of research that offers many opportunities for significant advances. It is already clear that some of the chemical transmitters and hormones that regulate neural activity can also directly affect the immune system. Glucocorticoids and catecholamines are known to influence autoimmune diseases, and corticosteroids are potent immunosuppressive agents currently used in the treatment of asthma and transplant rejection as well as autoimmune disease. The opioids may also play a role in immunoregulation, and stress mediators have been linked to changes in immune reactivity. Conversely, cytokines associated with inflammation and immune responses can modify neural cell function. Cytokines are possible mediators of behavioral changes during illness, and more work is needed to characterize the roles of individual cytokines in the brain. Largely uncharted areas include the functions of the immune system in inflammatory brain conditions, in infections and tumors of the brain, in degenerative neural diseases such as Alzheimer's, and in brain cell transplantation to correct neural defects.

Immune Response to Infectious Agents

Infectious diseases continue to plague the human populations of both developed and developing nations. Increasing worldwide travel and changing environmental conditions increase the risk of catastrophic global epidemics. In addition, the growing problem of resistance to antibiotics poses serious threats, and newly emerging and reemerging pathogens are of concern because immunity to them has not yet been established. The naïve immune system is able to combat many infections and generate immune memory that protects against repeat exposures to the same pathogens. However, some microorganisms can overwhelm or evade the immune system, leading to serious illness, irreversible tissue damage, and death. By evading or suppressing effective immune responses, certain viruses, bacteria, parasites, and fungi can persist for many years in human hosts without causing serious disease. Such latent infections can be transmitted unknowingly to susceptible individuals, and can be reactivated within the host to cause acute or progressively destructive disease. Furthermore, it is becoming clear that some latent infections are associated with cancer development. Thus, expanded studies on microbial pathogenesis and antimicrobial immune responses are needed to develop effective methods to eliminate pathogenic microorganisms.

Human pathogens constitute a broad spectrum of organisms that are both antigenically and biologically diverse. An even greater diversity of antigen receptors is expressed in lymphocyte populations, and the immune system is capable of recognizing most, perhaps all, invaders. However, immunity may be blunted or diverted by microbial products to prevent effective immune attack. In many cases, deliberate vaccination with microbial antigens under appropriate conditions can induce potent immunity and good protection. Thus, greater knowledge of the antigens and *in vivo* behavior of particular microbes, and a more detailed understanding of the types of immune

responses needed to destroy them, will facilite new vaccine development.

A number of new strategies for vaccine development are now being investigated, and exciting opportunities exist for ultimately conquering many devastating diseases. In addition to vaccines that prevent infection, therapeutic vaccines that can reverse established infections are also needed. Very few therapeutic vaccines have been developed to date. Their effectiveness may depend on activating different immunological processes than are induced by preventive vaccines. Advances in therapeutic vaccination would be of enormous benefit for the control of latent infections, for the containment of epidemics, and for the reversal of life-threatening infections at early stages of disease. Collaborative efforts among basic and clinical immunologists, microbiologists, geneticists, and vaccinologists will be important for rapid progress.

Novel Strategies for Vaccine Development

A number of effective vaccines were developed in recent years, but there is a continued need for new or improved vaccines. Enormous health benefits and economic savings can be realized by even one effective vaccine. HIV, malaria, tuberculosis, hepatitis C, and a variety of diarrheal and respiratory diseases are but a few examples of the many serious infectious diseases still to be conquered. Current research is focused on innovative approaches based on extensive advances in basic immunology and molecular biology. With increased knowledge of the type of immune response required to eliminate a particular pathogen, and improved understanding of immunoregulatory mechanisms, it should be possible to target specific immune components to induce robust protective immunity. New vaccine delivery systems are being devised that utilize recombinant DNA technology together with viral or bacterial vectors that express relevant microbial antigens in a context that will promote the most effective immune response. Recombinant antigen mixtures and non-infectious DNA vaccines that encode immunostimulatory cytokines, together with microbial antigens, are also being tested in animal models and clinical trials.

One major area of research is the construction of vaccines that target mucosal surfaces such as those in the intestine or respiratory tract. Mucosal vaccines are of great importance because many pathogens gain entry to the host *via* mucosal sites. Furthermore, oral vaccines are easy to administer and therefore of great utility in developing countries. The development of new adjuvants, which are compounds that enhance the stimulation of the immune system by antigens, is an important area of research that has progressed rapidly in recent years. A better understanding of normal immune responses in very young and aged individuals is needed to develop vaccines that protect these highly susceptible populations. It is known that the neonatal, pediatric, adult, and elderly immune systems differ, but detailed knowledge of specific differences in immune mechanisms is still lacking.

Genetic Basis of Disease Susceptibility

Susceptibility to all infectious and immune-mediated diseases is influenced by particular genes expressed in an individual. Extraordinary achievements in mapping the human genome have occurred over the past decade, and the basic sequences of all human genes will be known within the next decade. These accomplishments will allow identification of the multitude of genes that cause or modify disease progression. Future studies on the functions of proteins encoded by these genes will provide a vast body of new information important for the diagnosis, prevention, and treatment of a broad range of human diseases.

Perhaps the greatest challenge will be to evaluate the genetic contributions to the susceptibility and pathogenesis of complex diseases that are associated with more than one genetic locus. Work in this area has already begun, and it is now evident that many immune-mediated diseases, such as multiple sclerosis and type 1 diabetes, are influenced by a number of different genes. Multidisciplinary cooperative groups studying well-characterized affected families will utilize molecular approaches to define relevant genes and the functional consequences of their expression. Model systems that employ gene-altered animals will be important tools for sorting out potential roles of gene products in disease induction or pathogenesis. It will be imperative to develop comprehensive databases and programs for the ready analysis and interpretation of results. The genetic revolution has begun, and practical applications are already evident in the diagnosis and prognosis of certain diseases. Both basic research and medical practice will be profoundly influenced by the advances to come in the near future.

Nature of the Immune System

1

Overview

The mammalian immune system is composed of (1) white blood cells, or leukocytes (B and T lymphocytes, natural killer cells, monocytes, dendritic cells, neutrophils, basophils, and eosinophils), which circulate through the blood and lymph and enter and exit tissue compartments; (2) central, generative lymphoid organs such as bone marrow and thymus; and (3) secondary, peripheral lymphoid organs such as lymph nodes, spleen, and Peyer's patches. Defense against pathogenic infection results from two general types of cellular responses: the first involves constitutive, antigen-nonspecific mechanisms mediated by the "innate" immune system, and the second involves inducible, antigen-specific mechanisms mediated by the "adaptive" immune system.

The Innate Immune System

It has long been known that optimal antigen stimulation of adaptive immunity requires the use of auxiliary substances called adjuvants, which generally elicit antigen-nonspecific inflammation. It was recently realized that adjuvants act by engaging the innate immune system, which is thought to be the first line of defense against infection. Cells of the innate system, such as macrophages, dendritic cells, neutrophils, and epithelial cells, are found in skin and mucosal tissues that are frequent sites of microbial entry. These cells express constitutively a variety of invariant surface membrane or secreted receptors that bind lipid and polysaccharide structures found in microbes but not in mammalian cells. Thus, cells of the innate system can respond promptly to the presence of foreign microorganisms and be triggered to engulf and destroy pathogens or to secrete antimicrobial compounds. The complement system is also part of innate immunity (also see Chapter 5).

Importantly, a growing body of evidence suggests that products of the innate response provide strong signals to help activate adaptive responses. Thus, a variety of interactions occur between activated cells of the innate system and resting cells of the adaptive system to induce antigen-specific B and T cell responses. Among the products of innate responses are proteins called cytokines that help determine the particular type of adaptive response that ensues, and costimulatory cell surface molecules that are required for efficient T cell activation during specific T cell responses to antigen. Recent evidence that the innate immune defenses of insects such as drosophila use signal transduction proteins that are homologous to those used in mammalian immune responses indicates that the power of drosophila genetics may help shed light on basic mechanisms of mammalian immunity. The newly appreciated interplay between innate and adaptive responses provides a wealth of opportunities to target innate cell receptors with adjuvants that could greatly enhance the induction of appropriate antigen-specific adaptive responses to provide robust protective immunity.

The Adaptive Immune System

The remainder of this chapter focuses primarily on the adaptive immune system. The hallmarks of adaptive immunity are inducibility, specificity, memory, and the potential for immune tolerance. The essential cellular elements are the T and B lymphocytes. In each individual, the population of lymphocytes contains different cells that can recognize an almost limitless number of different antigens, and distinguish between those that are indigenous in the individual ("self") and those that are foreign ("nonself"). Lymphocytes comprise about 5 to 10 percent of all cells in the body, or about 10^{12} out of 10^{13} total cells in an adult human. The lymphocyte pool consists of an enormous number of different clones, each descended from a single progenitor cell and capable of recognizing only a few similar epitopes (antigenic determinants). The exquisite antigen specificity of these cells is due to cell surface protein receptors, with each clone having its own distinctive receptor. Thus, it is the collection of diverse receptor-bearing cells that provides protection against a broad spectrum of antigens.

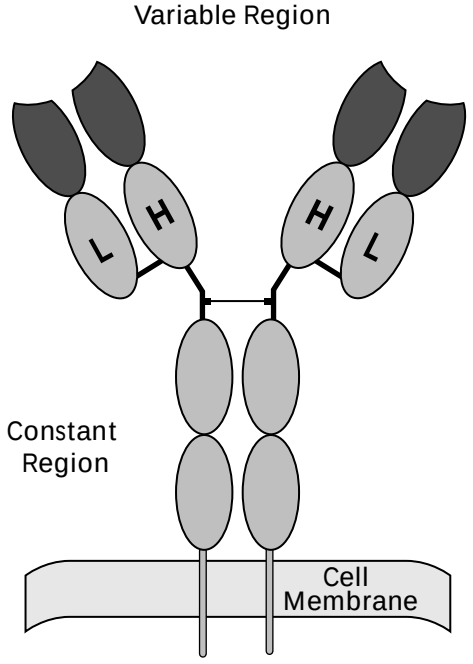

Figure 1-1. Immunoglobulin molecules are composed of heavy (H) and light (L) chains. They serve as antigen receptors when expressed on B cell surfaces and as antibodies when expressed in soluble form. Antigen binds to the variable region.

Source: Modified from Janeway, C.A., Jr.; Travers, P. (eds.). *Immunobiology: The Immune System in Health and Disease*. New York: Garland Publishing, Inc., 1997; used with permission.

About one-half of the lymphocytes, termed B cells, have immunoglobulin (Ig) receptors, also called B cell receptors (BCR), which are produced in soluble form as antibodies in response to BCR recognition of antigen under appropriate conditions (Figure 1-1). The other lymphocytes, called T cells, also recognize antigens through a cell surface antibody-like molecule, called the T cell receptor (TCR) (Figure 1-2). Unlike BCR, TCR are not secreted and they do not bind intact, free antigen. Rather, they react with cell surface major histocompatibility complex (MHC) class I or II molecules, but only when the MHC molecules bind antigen-derived peptides. Thus, the MHC proteins play a unique role in antigen recognition, serving to define those antigens that merit a T cell response. Since MHC proteins, with associated antigenic peptides, are found only on the surfaces of cells, T cells respond only to cell-bound antigen. Cytolytic T cells (CTL) respond by destroying the recognized cells. Reactions of this type provide defense against many viruses and other intracellular pathogens, and are also largely

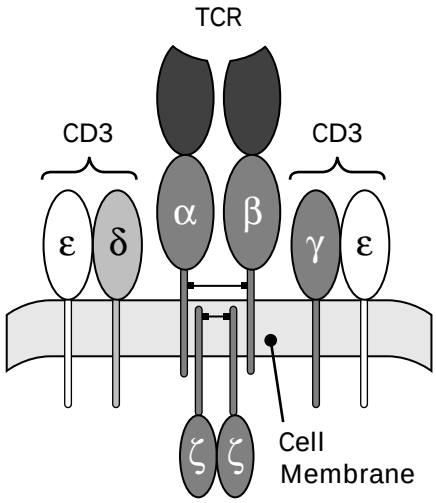

Figure 1-2. T cell antigen receptor (TCR) molecules bind antigen peptide-MHC complexes. TCR are associated with CD3 and ζ chains that mediate intracellular signal transduction.

Source: Modified from Janeway, C.A., Jr.; Travers, P. (eds.). *Immunobiology: The Immune System in Health and Disease*. New York: Garland Publishing, Inc., 1997; used with permission.

responsible for the destruction of tumor cells and transplanted organs.

Many of the vaccines that have been successful to date owe their primary effectiveness to the antibodies they elicit and to the long-term persistence of memory B cells capable of making high-affinity antibodies upon subsequent antigen exposure. Antibody production is often dependent on the interaction of B cells with CD4-positive helper T cells that are primed to the same antigen. However, for protection against many viruses, such as HIV-1, it appears that CTL, rather than antibodies, are required. Existing vaccines do not elicit sufficient production of CTL, but recent findings suggest that CTL may be generated by new DNA immunization protocols (see Chapter 14). The features of memory T and B cells that distinguish them from naive cells or from recently activated effector cells are not well understood. The extent to which memory cells are generated and persist following initial response to antigen is currently under intense investigation, because the goal of vaccination is to generate memory cells that will provide protection against subsequent exposure to the antigen.

A vast amount of information has been gathered in the past 15 years about the detailed structures of antibodies and, very recently, TCR; about the genetic mechanisms that underlie their production; and about how they recognize antigens and trigger B and T cell responses. Nevertheless, our understanding of how this remarkable and unique system actually functions in the body is still so fragmentary that it is only possible to regulate its activities to a limited extent. We are currently unable to create needed vaccines on demand or to deal effectively with many undesirable immune reactions such as autoimmunity or the rejection of transplanted organs or bone marrow. This difficulty is illustrated by our fight against AIDS. The causative virus, HIV-1, and its antigens are known, but we have yet to discover why anti-HIV-1 antibodies do not neutralize the virus and thereby protect against infection, or why virus-specific CTL or natural killer (NK) cells are not sufficiently effective to eliminate virus-infected cells. Thus, it has not yet been possible to design safe and effective vaccines to prevent or treat the infection. It is anticipated that continued research in basic immunology will provide these and other important answers to better

control the induction, regulation, and termination of immune responses.

Specific Antigen Recognition

B Cells and Antibodies

In the bone marrow, immature B cells develop the capacity to make BCR and antibody by specifically rearranging their Ig heavy and light chain V, (D), and J gene segments to create unique antigen receptors (Figure 1-3). A variety of different V, (D), and J segments are available in tandem regions on the chromosome, and different combinations of these segments result in different antigen-binding sites on each BCR. Mature B cells then leave the bone marrow and migrate to the periphery to await interaction with their antigen. After stimulation by antigen, a primary immune response develops during which antibody-secreting antigen-specific plasma cells and memory B cells form. Somatic mutation in the Ig variable region provides even greater diversity and opportunities for antigen to preferentially activate the proliferation and persistence of those B cells that can produce higher affinity antibodies than produced in the initial population. Upon subsequent antigenic stimulation, often years after the initial response, a secondary immune response ensues and antibodies of high affinity are produced by memory cells, making them especially effective in host defense. Somatic mutation of the Ig variable gene segments occurs 10,000 times more frequently than in other genes; hence this process is called somatic hypermutation.

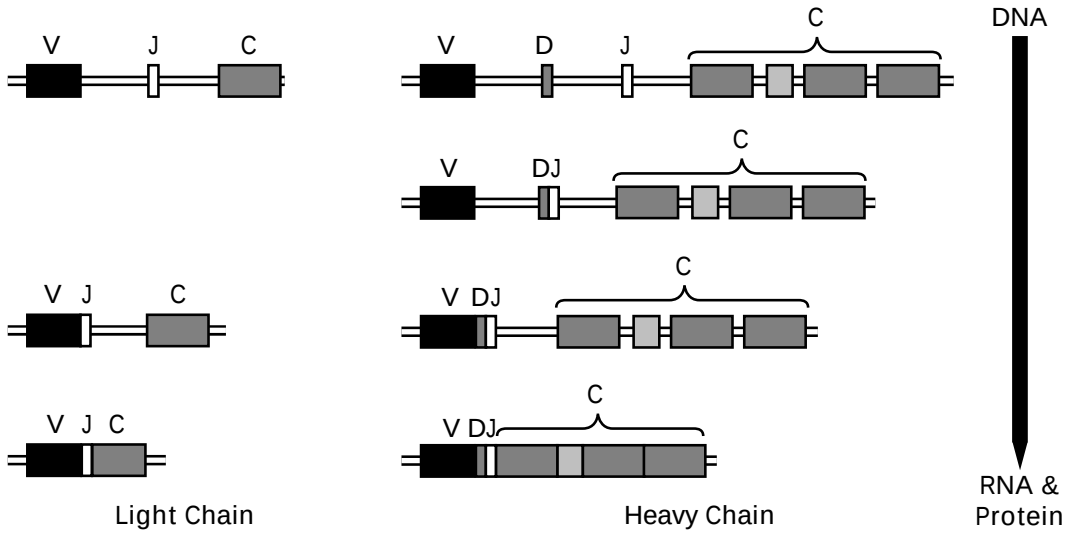

Figure 1-3. Within a particular B cell, the immunoglobulin genes rearrange to include only one of many possible V (variable), D (diversity), and J (joining) gene segments to create a unique antigen specificity.

Source: Modified from Janeway, C.A., Jr.; Travers, P. (eds.). *Immunobiology: The Immune System in Health and Disease*. New York: Garland Publishing, Inc., 1997; used with permission.

During the rearrangement of Ig V, (D), and J genes, as well as during somatic hypermutation, it is likely that antibodies with specificity for self antigens will develop. For prolonged good health we must have a mechanism for destroying these autoreactive cells. To understand these mechanisms we first need to understand the mechanisms by which antibodies develop normally. Major advances in recent years include the development of a cell-free system to study the mechanism of V(D)J gene recombination and the discovery that the lymphoid-specific enzymes, Rag-1 and Rag-2, and the more ubiquitous enzyme, DNA-PK, play crucial roles in V(D)J gene recombination. Recent excitement also centered on the discovery that germinal centers are the sites where Ig genes hypermutate and where B cells producing antibodies of high affinity are selected. Germinal centers are specialized areas that develop in the peripheral lymphoid organs during immune responses.

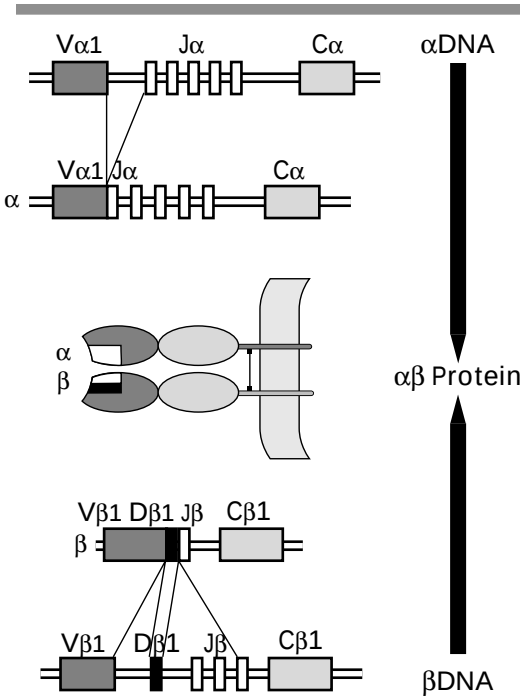

Figure 1-4. Within a particular T cell, the TCR α and β genes rearrange in a manner analogous to immunoglobulin genes to create a unique specificity for peptide-MHC binding.

Source: Modified from Janeway, C.A., Jr.; Travers, P. (eds.). *Immunobiology: The Immune System in Health and Disease*. New York: Garland Publishing, Inc., 1997; used with permission.

T Cells

Like B cells, T cells first express genetically rearranged antigen receptors (TCR) at an immature stage (Figure 1-4), and the same enzymatic machinery is used by B and T cells in rearranging variable gene segments for the generation of receptor diversity. For recognition by T cells, antigens are first degraded into short peptides of 8 to 15 amino acids that bind to MHC proteins for display on the surface of antigen-presenting cells (APC), also called target cells. The specificity of TCR recognition usually depends on contact with both the peptide and the MHC molecule. T cell surface CD4 and CD8 molecules act as coreceptors and play a role in recognition. During TCR engagement by peptide-MHC complexes, CD4 or CD8 proteins bind to the MHC II or I molecule, respectively, and help stabilize the TCR:peptide-MHC interaction. In addition, signals mediated by the ligated coreceptor contribute to the intracellular cascade of activation events initiated by the TCR. Interestingly, peptide-MHC complexes are generally bound to TCR with lower affinities than most antigens are bound to antibodies. Moreover, in contrast to Ig, TCR do not hypermutate during antigen activation. Thus, although T cell specificity is exquisitely dependent on TCR, normal activation of the T cell

generally requires the contributions of CD4/CD8 as well as other invariant accessory molecules that increase cell:cell adhesion or provide costimulatory signals.

Lymphocyte Repertoire Selection

Antigen receptors are produced in developing B cells without an obvious bias for specificity. The same is true for T cells except for the propensity of TCR to bind MHC proteins weakly. Cells are selected from this large potential repertoire for the ability to combat pathogens without harm to the individual. Selection processes are triggered by specific antigen recognition and are critical for shaping the foreign antigen-specific, self antigen-tolerant repertoire of mature lymphocytes.

B Cells

The early maturation of B cells depends on expression of a rearranged Ig heavy chain complexed with invariant surrogate light chains. It is thought that this pre-BCR delivers a survival and maturational signal, thus inducing a form of positive selection. However, pre-BCR ligands have not been identified thus far. Maturation of B cells may be blocked at several checkpoints by antigen recognition, thus helping to ensure that self-reactive mature B cells do not develop. After maturation, foreign antigen stimulation induces B cells to enter germinal centers in lymphoid follicles and undergo hypermutations in variable domains of the BCR. Antigens displayed by follicular dendritic cells selectively stimulate B cells with high-affinity BCR to proliferate and develop into antibody-secreting plasma cells or into quiescent, long-lived memory B cells. This selection process is responsible for the affinity maturation of antibody responses and, thus, an improved ability to generate antibodies that bind the specific antigen at low concentrations. Because hypermutation might generate new self-reactive B cells, mechanisms exist to inactivate or kill such cells even at the mature stage, or to change the Ig specificity by "receptor editing" (see Chapter 2). The mechanisms that destroy self-reactive cells but preserve foreign antigen-reactive cells are not well understood.

αβ T Cells

Most T cells mature in the thymus and express αβTCR. In these cells, the β genes are assembled first and are expressed at an early stage in "double negative" (CD4- and CD8-negative) thymocytes. Owing to combinatorial variation in the assembly process, the β chains of each cell differ from one another in the sequence of their antigen-combining regions. Despite this variation, a T cell's β protein will then pair with an invariant pre-Tα protein. This forms a pre-TCR capable of transmitting a signal that stimulates the cell to proliferate and advance to the next stage, in which both CD4 and CD8 proteins are expressed on the cell surface ("double positive" thymocytes). Then, TCR α genes are rearranged, and the resulting α protein replaces the pre-Tα to form a mature αβTCR. It is at this point in development that peptide-MHC complexes can be recognized and positive and negative selection can occur. It was recently shown that even the unselected αβTCR repertoire is biased toward recognition of MHC proteins. Cells are selected to survive and mature into single

positive (CD4 or CD8) T cells if the self peptide-MHC complexes recognized on the thymic epithelium are not too strongly stimulatory; other cells die. Within the positively selected pool of immature T cells, those that react too strongly to self peptide-MHC complexes are deleted by apoptosis. This process, termed negative selection, helps eliminate T cells that could react too aggressively with self antigens. The particular stimuli and biochemical signals that control positive and negative selection are not yet defined.

T cells that are capable of recognizing self antigens present in peripheral tissues but not in the thymus can mature and populate peripheral tissues. Thus, as with B cells, peripheral mechanisms exist to inactivate mature, potentially aggressive T cells that recognize self antigens. Much recent work has focused on identifying the physiological mechanisms that regulate peripheral T cell tolerance (see Chapters 8 and 9). Although considerable progress has been made, it is not yet known which mechanisms are the most effective in maintaining physiological self tolerance, or which might be exploited most effectively to inhibit unwanted responses to foreign antigens, such as allergens or transplanted organs, or to restore tolerance in autoimmune disease.

γδ *T Cells*

A second class of T cells expresses another type of heterodimeric TCR, formed of γ and δ proteins. The biological role of γδ T cells is not as well understood as that of the αβ cells, although much has been learned in recent years. In mice, they appear to help protect against certain infectious diseases and to ameliorate some autoimmune diseases. The ligands that are recognized by γδTCR differ considerably from the peptide-MHC complexes seen by αβTCR, and include organic pyrophosphates of bacterial and mycobacterial origin, and some proteins, such as CD1, which is a MHC-like protein that presents glycolipids, not peptides. The γδ T cell repertoire does vary in different tissues and at different stages of development, but the role of specific selection processes is unknown.

NK Cells

Most NK lymphocytes do not express clonally distributed, rearranged antigen receptors like T and B cells. Interestingly, they bear MHC class I-reactive receptors that are distinct from TCR and that *turn off* NK cell cytolytic activity when MHC I is recognized. These NK receptors are synthesized from a family of related genes that do not rearrange, and different receptors having overlapping specificities for various MHC I proteins are distributed on different NK clones; one NK cell can express more than one receptor. Thus, NK cells have a complex repertoire of MHC receptors, and different members of the receptor family discriminate among different allogeneic MHC molecules. When bound by MHC, these receptors inhibit the natural cytotoxic function of NK cells that will lyse MHC I-negative transformed or virally infected cells. Many cancer cells and virus-infected cells downregulate MHC I surface expression to evade recognition and destruction by CD8 T cells. By specifically destroying cells that lack appropriate MHC I proteins, NK cells can overcome these viral and tumor evasion mechanisms. It is not yet known how the NK cell repertoire is

formed or how self tolerance is established.

Molecular Basis of Lymphocyte Activation

The most important recent advances in lymphocyte activation are the delineation of (1) signal transduction mechanisms mediated by BCR, TCR, and cytokine receptors; (2) the role of intracellular protein phosphorylation in the regulation of lymphocyte activation; (3) the basis for transcriptional regulation of cytokine gene expression; (4) the regulation of cell death by apoptosis; and (5) the realization that the TCR is not an on or off switch, but rather can turn on graded responses and even qualitatively different T cell responses to certain peptide-MHC ligands.

Specific lymphocyte antigen receptors associate noncovalently with a set of membrane-spanning, clonally invariant proteins: the CD3 γ, δ, and ε chains and the ζ chains on T cells, and the Igα and Igβ chains on B cells (Figures 1-2, 1-5, 1-6). Each of these receptor-associated

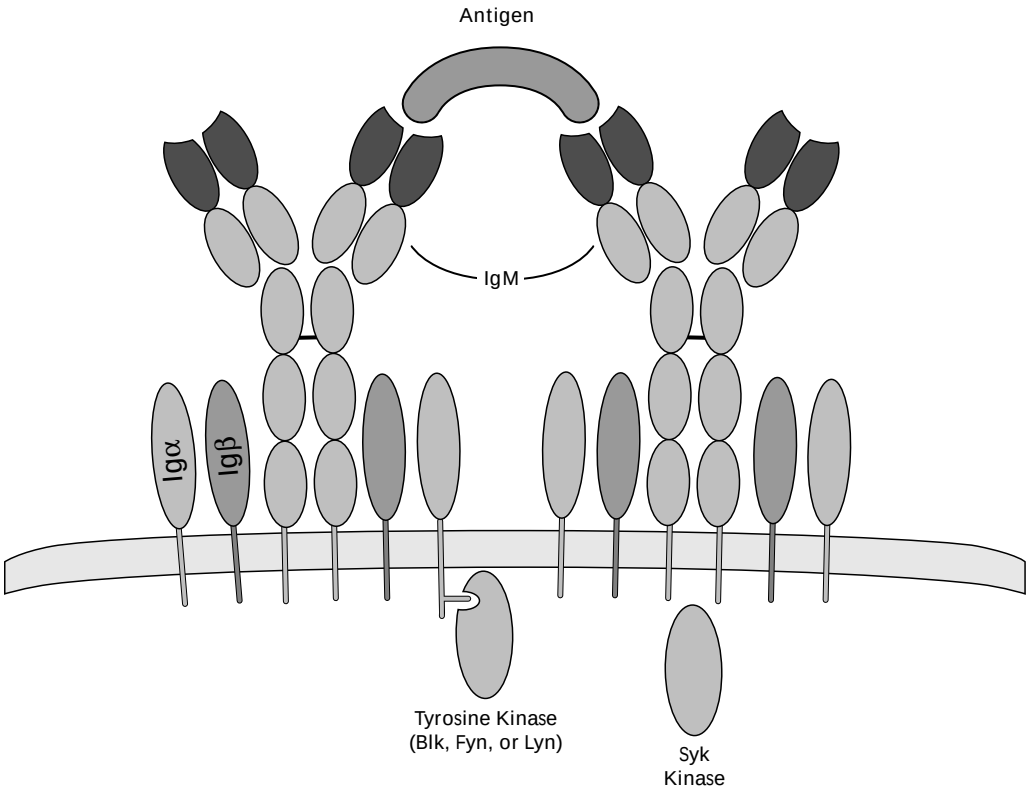

Figure 1-5. Surface IgM is associated with other molecules in the cell membrane that mediate activation of tyrosine kinases to initiate signal transduction upon antigen crosslinking.

Source: Modified from Janeway, C.A., Jr.; Travers, P. (eds.). *Immunobiology: The Immune System in Health and Disease*. New York: Garland Publishing, Inc., 1997; used with permission.

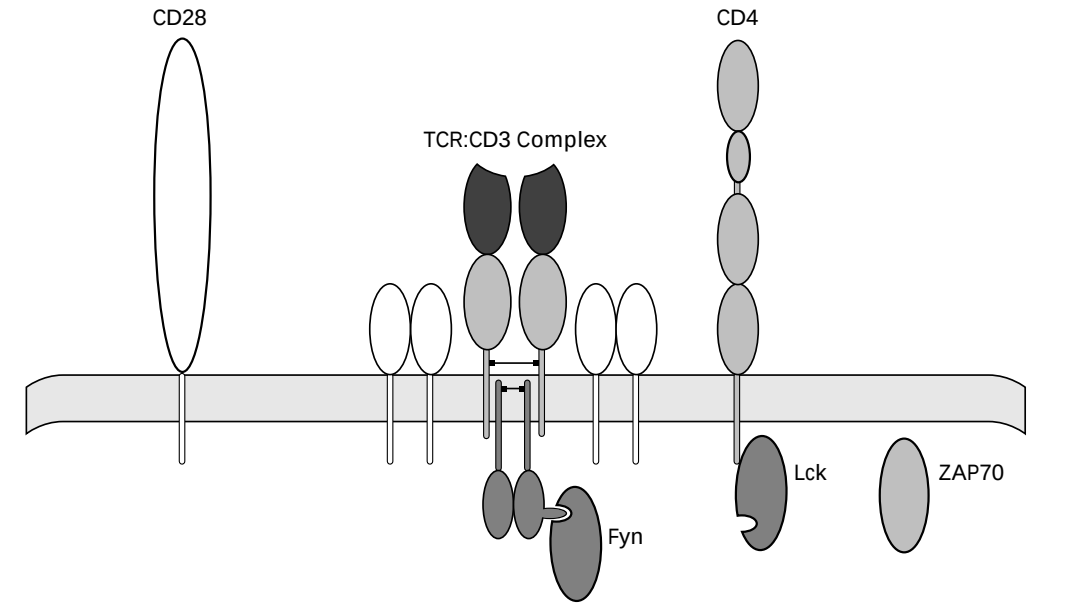

Figure 1-6. T cells usually require CD4-MHC (or CD8-MHC) and CD28-B7 interactions, in addition to TCR: peptide-MHC interactions, for full stimulation. As in B cells, tyrosine kinases initiate the cascade of intracellular signals that turn on new gene transcription.

Source: Modified from Janeway, C.A., Jr.; Travers, P. (eds.). *Immunobiology: The Immune System in Health and Disease*. New York: Garland Publishing, Inc., 1997; used with permission.

proteins contains one or more copies of a cytoplasmic "ITAM" (immunoreceptor tyrosine-based activation motif), which is a target for specific tyrosine phosphorylation. Cascades of biochemical events are induced upon antigen binding to the receptor, with perturbation of the balance of protein tyrosine phosphorylation by changes in kinase and phosphatase activities.

Sets of receptor-associated intracellular protein tyrosine kinases—the Lck, Fyn, and ZAP70 kinases in T cells and the Lyn, Blk, Fyn, and Syk kinases in B cells—were found to mediate these responses. At least one function of the coreceptors CD4 and CD8 on T cells and CD19/CD21/TAPA on B cells is to localize these kinases in proximity with the receptor ITAMs, thereby increasing the efficiency of receptor signaling. For example, it was demonstrated that CD4-associated Lck can act to phosphorylate the ITAM motifs of the CD3 and ζ proteins, thereby providing binding sites for ZAP70, which itself becomes activated through phosphorylation and acts on proteins further downstream in the signaling cascade. A coherent model of the early steps in T cell activation has emerged from such studies, and similar progress has occurred in understanding BCR cell signaling.

Cytokine receptors consist of two or more chains that interact specifically with and activate cytoplasmic protein tyrosine kinases of the Jak family. These kinases,

in turn, contribute to phosphorylation of the receptor chains that promotes the recruitment of various signaling molecules, such as the Shc coupling protein, which is involved in Ras regulation, and STAT proteins, which become phosphorylated and translocate to the nucleus where they regulate gene transcription.

The consequence of antigen or cytokine stimulation is the transcriptional activation of target genes that encode inducible surface signaling molecules or soluble immune mediators such as cytokines or that move the cell into cycle for proliferation, inhibit a cell death program, or even induce anergy. Many of the transcription factors involved in these responses have been identified, although considerable work remains before these molecular events are fully understood. Among the transcription factors known to be involved in lymphocyte gene regulation are members of the NF-AT, AP-1, Rel, and STAT protein families.

Recent work has begun to define the molecular events that determine whether an antigen-stimulated lymphocyte will undergo clonal expansion or activation-induced cell death or will be rendered anergic to subsequent stimulation. Critical roles for the Bcl-2-related proteins and proteins that regulate the caspase proteases during apoptosis have been implicated in the life-or-death decisions. It is remarkable that a single antigen receptor, ligated by variant antigenic forms or under different costimulatory conditions, can provoke a wide variety of responses. The type of response can depend on whether a cell is immature or mature or whether a mature cell is of the naive or memory phenotype. Many opportunities now exist to define further the underlying regulatory pathways, with the goal of predictably changing B or T cell fates in an antigen-specific manner for therapeutic benefit in humans.

Mechanisms of Lymphocyte Effector Functions

B Cells: the Humoral Response

The principal effector molecules produced by B cells are antibodies. Antibodies function by neutralizing viruses and toxins, by activating the complement system, and by activating effector cells such as phagocytes, NK cells, and mast cells through their Fc receptors, which bind the constant regions (Ig Fc) of antibody:antigen complexes. For example, antigen cross-linking of IgE bound to the FcεRI receptor on mast cells results in the release of inflammatory and vasoactive mediators that can give rise to life-threatening allergic reactions. Among many advances, we highlight the molecular characterization of FcεRI for its potential in developing therapy for allergic diseases. This receptor has three protein subunits, α, β, and γ. The α subunit binds IgE while the β and γ subunits transduce the signals that cause the synthesis and secretion of allergic mediators. Characterization of this receptor provides the opportunity to develop therapy for allergic diseases by interrupting the binding of IgE to the α subunit or by suppressing the signaling through the β and γ subunits.

Recent observations have focused on the impact of molecules associated with the innate immune system, such as comple-

ment, on the adaptive immune system. Analysis of the complement receptor, CR2, on B cells showed that it associates with another membrane protein, called CD19, and that antigens bound to the complement component, C3d, coligate the BCR, CD19, and CR2 to enhance greatly the B cell response; remarkably, 1,000- to 10,000-fold more antibody was produced than in the absence of C3d. These observations provide the opportunity to develop more effective vaccines for infectious diseases.

T and NK Cells: the Cellular Response

Three major T and NK cell effector functions have been defined in detail in the past 10 years: cytotoxicity, macrophage activation to kill pathogens, and provision of help to B cells to produce antibody. These functions can be explained to some extent by polarized cytokine secretion, induction of surface membrane ligands that interact with target cells, and molecules carried in the specific lytic granules of CTL and NK cells. Gene knockout mice have been produced that are defective for expression of many of these effector molecules, such that a comprehensive picture of the effector arm of T cell adaptive immunity is beginning to emerge.

CTL are generally CD8 T cells that recognize peptide-MHC I complexes. Because most somatic cells express MHC I, CTL can kill many different target cells, including cells infected with viruses and cancer cells. CTL bind to target cells and release lytic granules in a polarized

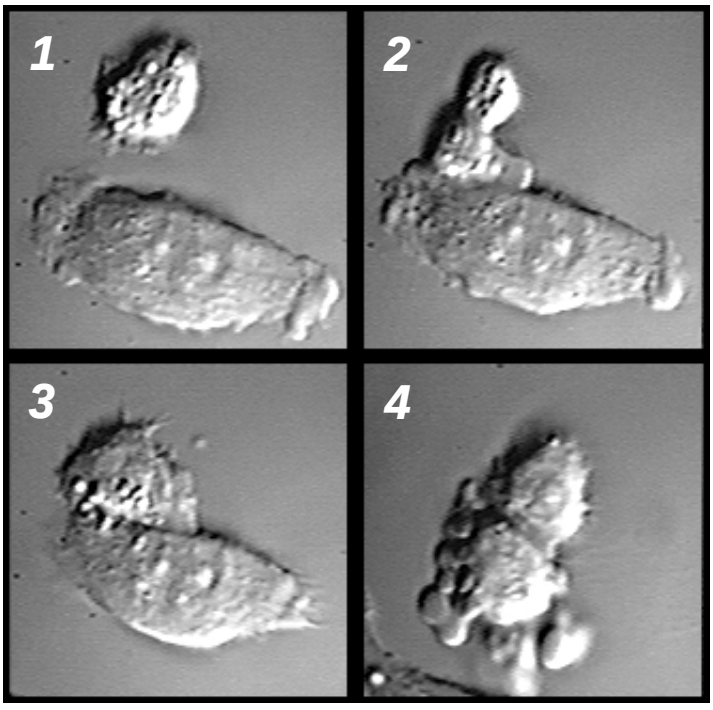

Figure 1-7. Killing by Allogeneic CTL. H-2^d anti-H-2^b allogeneic CTL were added to H-2^b target cells, and killing was studied using VEC-DIC microscopy. This sequence of images illustrates specific stages of the killing process. Initially, random ruffling and extension of small lamellae around all sides of the CTL ceased, and a single large lamella extended toward the target (frame 1). Large granules in the cytoplasm of the CTL moved to the point of CTL-target cell contact (frames 2-3). During granule movement, the CTL spread along the edge of the target cell, maximizing contact between them (frames 2-3). The target then contracted and blebbed as the granules disappeared at the CTL-target interface (frames 3-4). The CTL left the target while blebbing continued, after contraction was near maximum. Often the CTL killed additional targets. Morphological changes in both the CTL and target cells were indistinguishable from those seen for virus-specific CTL, and the kinetics of killing were similar.

Source: Modified from Hahn, K.; et al. Antigen presentation and cytotoxic T lymphocyte killing studied in individual living cells. *Virology* 201:330–340, 1994; used with permission.

fashion onto the target cell surface (Figure 1-7). Among the granule proteins is perforin, which forms channels in the target cell membrane, allowing granzymes to enter and activate caspase enzymes in the target cell. Perforin deficiency is associated with severe defects in antiviral and antitumor defense, and granzyme deficiencies cause an impairment in apoptosis, although lysis can still occur by an as yet unidentified mechanism. NK cells utilize the same perforin/granzyme mechanism to kill cells that have lost surface expression of classical MHC class I proteins.

A second method of killing, mediated by both CD8 and CD4 T cells, involves the binding of Fas ligand (FasL) on the T cell to Fas on the target cell. FasL is induced on T cells during activation; its binding to Fas triggers apoptosis in the target cell *via* the caspase enzyme cascade. FasL-dependent cytotoxicity is implicated in a growing number of diseases. It is now clear that defects in Fas or FasL can result in the failure to eliminate self-reactive T cells and, thus, in autoimmune disease. The T cell-produced cytokine TNF and its receptors (TNFR) constitute a third cytotoxic pathway. TNF and TNFR are related to the Fas pathway but act with slower kinetics and less restricted action. TNF is thought to play an important role in chronic inflammatory diseases such as rheumatoid arthritis and multiple sclerosis, and newly developed anti-TNF compounds are showing promise as effective therapeutic agents in human trials.

The discovery that CD4 T cells differentiate into functionally distinct subsets upon antigen activation provides a useful paradigm for analyzing particular types of immune responses and interesting opportunities to externally manipulate antigen-specific immunity. CD4 cells recognize peptide-MHC class II complexes, which are normally expressed on only a few cell types. Thus, CD4 activation is thought to orchestrate immune responses by responding to "professional" antigen-presenting cells that signal the presence of a foreign antigen. The major CD4 subsets are designated Th1 and Th2, based on the mixture of cytokines they secrete upon activation, and more recently, on differential surface expression of certain proteins (also see Chapter 4). Activated Th1 cells secrete IL-2 and IFNγ and express greater cell surface levels of FasL than CD40 ligand (CD40L). In contrast, Th2 cells produce IL-4, IL-5, IL-10, and IL-13 and express more CD40L than FasL. CD40 on B cells, macrophages, and dendritic cells binds its counterreceptor, CD40L, and mediates activation of these APC.

Th1 responses often lead to macrophage activation for pathogen killing, generate inflammation, and stimulate production of particular antibody isotypes. Th2 responses are characterized by high levels of antibody production, including the preferential secretion of IgE. Although this subdivision of CD4 cells is likely to be an oversimplification, it has served to demonstrate that functionally distinct effector CD4 cells can be generated during an immune response, that the type of CD4 response depends on the cytokine milieu of the activation conditions, and that the dominance of one subset over another can dictate whether the immune response is appropriate to protect against the pathogen. These recently developed

concepts provide a wealth of opportunities for antigen-specific modulation in clinical applications.

Leukocyte Migration

The continuous circulation of lymphocytes through the blood and lymphatic system to various lymphoid organs is essential for host defenses because it increases the chance that specific lymphocytes will encounter their antigen. Furthermore, the migration of leukocytes to sites of injury or infection is important in inflammatory responses. Studies during the early 1980s revealed (1) the existence of tissue-selective mechanisms of lymphocyte:endothelial cell recognition, offering an explanation for selective lymphocyte trafficking to certain organs or tissue sites, and (2) the discovery of cytokine regulation for leukocyte recruitment at sites of inflammation. Assays were developed that, in combination with specific monoclonal antibodies, allowed the identification of leukocyte:endothelial cell adhesion molecules, leading to the molecular cloning of some of the major players by 1992. It then became clear that simple models of leukocyte:endothelial cell recognition were inadequate, and seminal work demonstrated that separable cell rolling and β_2-integrin-dependent sticking events occur (see Chapter 5, Figure 5-1). Thus, a model including multiple steps was developed that provides for the combinatorial determination of specificity and diversity in leukocyte:endothelial cell recognition and extravasation. One of the important consequences of this generalized multistep model was that it brought together previously unrelated studies of adhesion/homing receptors and leukocyte chemoattractants. Work in the 1970s and 1980s had emphasized the ability of classical chemoattractants to trigger neutrophil aggregation and directed migration. In the past several years, this paradigm was extended to lymphocytes, resulting in the emerging characterization of chemoattractant receptors and identification of some of their ligands. One major recent technical advance was the development of *in situ* videomicroscopic methods to visualize and dissect leukocyte:endothelial interactions, and *in vitro* models of such interactions have contributed to an appreciation of the sophisticated molecular specialization involved. In addition, the ready construction of transgenic and gene knockout mice has allowed the study of leukocyte migration and microenvironmental homing *in vivo*, and such models will continue as important research tools.

Mucosal Immunity

Despite enormous advances in understanding immunity at the systemic level, knowledge of the mucosal immune system is relatively sparse. It is known that mucosal and systemic responses can differ dramatically, and it is important to remember that the majority of infectious agents enter the body through mucosal surfaces in the respiratory tract, intestines, and reproductive tract. Yet, the body must distinguish between potential pathogens and a continuous barrage of innocuous environmental and food antigens. In fact, it has been known for many decades that the oral administration of antigen can result in the generation of systemic unresponsiveness to that antigen (oral tolerance). Paradoxically, it is also well established that oral administration of infectious agents, such as polioviruses, can elicit potent systemic immunity. The mechanisms responsible for the divergent

outcomes are still obscure. When these mechanisms are well understood and can be manipulated at will, and the responsible antigens are identified, the ability to elicit oral tolerance consistently holds the promise of inexpensive and simple treatment for autoimmune diseases and allergies, whereas predictable immunization by mucosal routes will allow the production of more effective vaccines.

Continued investigations into mucosal immune regulation and improved vaccine delivery systems are essential for harnessing the potential of the mucosal immune system to protect against a wide variety of infectious organisms. For example, infection with HIV most often occurs through the vaginal or rectal mucosa, and mucosal immunity is likely to play an important role in the development of a protective vaccine for HIV. IgA is the predominant antibody isotype associated with mucosal surfaces, and recent accomplishments include characterization of the mechanism that transports IgA across the mucosal epithelium and the identification of TGFβ and IL-5 as cytokines involved in B cell switching to IgA. Other advances include the phenotypic and functional characterization of intraepithelial lymphocytes; the migratory pathways of mucosal lymphocytes and identification of an integrin molecule that is essential for homing of lymphocytes to gut-associated lymphoid tissues; and the design of experimental vaccines and antigen delivery systems for inducing mucosal immune responses and mucosal tolerance.

Advances in Methodology

The identification of cell type-specific markers and the generation of numerous specific antibody reagents in recent years have led to greatly improved detection of single cell activation events and to the discrimination of responses by different immune cell types. Furthermore, new methods developed in molecular biology have revolutionized the ability to detect and manipulate immune-related proteins in highly specific and sensitive assays. The topological redistribution of antigen receptors and costimulatory molecules on the cell surface can now be followed by high-resolution fluorescent microscopy during cell activation. The ability to express integral membrane proteins as soluble molecules has made it possible to analyze the affinity and kinetics of TCR interactions with peptide-MHC complexes. In addition, the crystal structures of several TCR:peptide-MHC complexes have been solved, allowing detailed structural comparisons between the basic elements of B cell and T cell immunity. Of particular interest are recently developed methods to engineer soluble peptide-MHC complexes for the sensitive detection of antigen-specific T cells within populations. Thus, a specific immune response can be monitored in different tissues and at different times after immunization in normal animals. The expression of fluorescent proteins in transgenic mice adds to the exciting new areas for future research in the ongoing development of sophisticated imaging technology to visualize specific B and T cells in intact tissues and to follow their migration and responses in intact animals.

Transgenic mice engineered to express a new protein of choice, and gene knockout mice in which a normal cellular gene is made nonfunctional, have proved to be extraordinarily valuable tools for explor-

ing the immune system. Many of the chapters in this report include a discussion of the utility of these unique mouse models. Studies of transgenic mice carrying Ig, TCR, MHC class I and class II genes, as well as foreign antigen genes, have increased understanding of thymic T cell selection, B cell development, and immune tolerance. Of immediate practical interest, transgenic mice that carry the human Ig gene repertoire have been produced recently. These mice provide the opportunity to develop monoclonal human antibodies of virtually any desired specificity for immunotherapy, without the disadvantages encountered using mouse antibodies in humans. With the use of phage display "libraries," it also appears that high-affinity human antibodies may be produced *in vitro* utilizing lymphocytes from infected or immunized individuals.

Knockout mice have been used extensively to study the function of specific genes. For example, the Rag-1 and Rag-2 knockout mice have no B or T cells, and study of these mice revealed that Rag-1 and Rag-2 are essential for V(D)J gene rearrangements. These mice have also provided the opportunity to study the function of lymphoid-expressed genes that are also essential for survival, such that mice homozygous for the nonfunctional gene die early in development. In this case, blastocysts of Rag-1 or Rag-2 knockout (host) mice can be injected with embryonic stem cells carrying the nonfunctional gene of interest and intact Rag genes. In the resulting chimeric mice, the Rag-deficient host cells can compensate for the nonfunctional gene in all cells except lymphocytes, since all lymphocytes in these animals will arise from the embryonic stem cells having the nonfunctional gene; thus its effect on lymphocyte development can be examined. Another major advance in gene-targeting technology is the ability to excise genes only in specific tissues and at particular stages of differentiation by using the Cre-loxP system (see Chapter 15, Figure 15-2).

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Research Opportunities

Immune Activation

- Determine how engagement of the innate immune system promotes activation of adaptive immunity
- Characterize the activating and inhibitory receptors of NK cells
- Elucidate the signal transduction pathways in lymphocytes from receptor engagement to activation of transcription factors that regulate gene expression

Lymphocyte Antigen Receptor Diversity

- Determine the precise mechanisms of Ig V(D)J recombination and the factors that regulate gene recombination
- Identify the mechanisms of B cell somatic hypermutation

Lymphocyte Repertoire Selection

- Define the signals that allow newly developed B cells to survive
- Identify the mechanisms responsible for affinity selection of B cells following somatic mutation
- Characterize ligands recognized by γδ T cells and determine how the γδ T cell repertoire is selected

Mucosal Immunity

- Identify the cells and cytokines involved in oral tolerance; determine whether mucosal epithelial cells participate in the activation or downregulation of immune responses to common environmental antigens and how these cells interact with mucosally administered vaccines
- Develop optimal forms of immunogens and methods of immunization to induce mucosal immunity and long-term immune memory
- Determine whether the genital mucosa is part of the common mucosal immune system and whether genital immunization protects against sexually transmitted diseases such as HIV, chlamydia, papillomavirus, and gonorrhea
- Define the role of γδ T cells in protecting the integrity of mucosal surfaces

Leukocyte Migration

- Define the adhesion/activating/ chemoattractant cascades of molecular events that target leukocytes in clinically important disease states to allow selective, therapeutic inhibition of recruitment or inflammation
- Investigate the regulation of leukocyte trafficking in specific microenvironments within tissues
- Identify the intracellular signaling pathways involved in chemoattractant and adhesion regulation of leukocyte:endothelial cell interactions

Translation of Basic Research into Clinical Applications

- Apply insights from *in vitro* studies and animal models of immune dysfunction to better understand the human immune system
- Develop clinically applicable assays to measure antigen-specific human T cell responses to vaccines and to monitor specific T cell reactivity during infection, allergic responses, autoimmune disease, allotransplant rejection, and cancer development

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Development of the Adaptive Immune System

2

Overview

Considerable advances have occurred over the past 10 years in understanding the mechanisms that regulate each stage of immune development. The cellular components of the immune system have limited life spans and must be replaced continuously with new cells. This process begins in the fetus, continues throughout adult life, and involves a series of complex differentiation steps in precursor cells that define the functional capacities and longevity of the mature cell subsets. This regenerative property provides extraordinary plasticity and a strong advantage in circumstances of transient immune depletion. This chapter focuses primarily on lymphocyte development, but many of the issues raised apply equally well to very important questions on the development of the innate immune system and of antigen-presenting cells such as macrophages and dendritic cells.

A wealth of information important for many clinical applications has been provided by the identification of precursor, intermediate progenitor, and mature cell phenotypes; stimulatory and inhibitory cytokines; stromal and hematopoietic cell receptors for growth; differentiation and migration signals; intracellular signal transduction pathways; and transcription factor regulation. Applications of such research advances can be seen in bone marrow transplantation, anemia, leukemia, and immunodeficiency diseases. Clearly, however, much remains to be learned, and available technologies provide many opportunities to define the cellular, genetic, and biochemical parameters of immune development at the more detailed level required for accelerated translation of basic research to human diseases. In particular, more work is needed on the development of the human immune system, which is known to differ in some respects from animal models. Immune development in the human neonate is not well understood, and very little is known about the immune capability of premature infants. Approximately 10 percent of infants in the United States are born prematurely, many at less than 34 weeks of gestation, and they are at greatest risk for pneumonia, meningitis, and nosocomial infections.

Hematopoietic Stem Cells and Lineage Commitment

The hematopoietic stem cell (HSC) is the ultimate precursor of lymphocytes, conventional antigen-presenting cells, and cells of the innate immune system, as well as red blood cells and megakaryocytes, or platelet-producing cells (Figure 2-1). Remarkably, at the single-cell level, HSC can both self-renew and give rise to progeny that differentiate into multiple cell types. Multipotent or pluripotent progenitor cells are the intermediates between the HSC and those progenitor cells that are committed to a defined lineage. Although the identity and hierarchical relationships of the multipotent progenitors remain to be fully elucidated, it

is known that progression through the hematopoietic cascade involves a tightly controlled pattern of gene expression that is orchestrated by a complex set of proteins called transcription factors.

Analysis of transcriptional regulation of lineage-specific genes, pioneered by studies on immunoglobulins and globins, has led to the characterization and cloning of various cell type-specific transcription factors in the hematopoietic system. Recently, gene knockout technology in mice has been used to examine the functions of these transcription factors during hematopoiesis. For example, mutations in genes encoding the transcription factors GATA-1, c-myb, PU.1, Ikaros, E2A, EBF, Pax5, Sox-4, TCF-1, GATA-3, NF-E2, and C/EBPa have all been shown to result in lineage-specific developmental defects of the hematopoietic system. Loss of c-myb function results in erythroid, myeloid, and lymphoid defects; loss of PU.1 results in myeloid and lymphoid defects; and loss of Ikaros results in lymphoid lineage defects. These phenotypes indicate that c-myb, PU.1, and Ikaros initially function within multipotent progenitors. Furthermore, their apparent order of action has been used to suggest a hierarchical scheme that invokes ordered and sequential generation of lineages in the hematopoietic system. This scheme differs from the classical model of hematopoiesis in that lineage-committed progenitors arise in a developmentally ordered manner, with the lymphoid and myeloid lineages being generated from a common multipotential progenitor.

In contrast to c-myb, PU.1, and Ikaros, the combination of E2A, EBF, Pax-5, and Sox-4 is specifically required for B lymphocyte development, and TCF-1 and GATA-3 are required for T lymphocyte development. The existence of unique combinations of transcription factors that are necessary for the development of specific lineages strongly suggests that lineage commitment decisions are not regulated by single "master" genes but rather by distinct combinations of regulatory genes.

Although the gene knockout approach has provided a powerful means to define sets of transcription factors required for the generation of lineage-committed progenitors, it has several limitations. It establishes the requirement for a given transcription factor in lineage commitment, but not its sufficiency, which can only be demonstrated by gain-of-function or ectopic expression experiments with the relevant transcription factor. Thus, experiments involving constitutive or inducible expression of these transcription factors in multipotent or inappropriate progenitors will be needed to determine whether they can induce or redirect lineage commitment decisions. Furthermore, there may be redundant sets of regulators that can replace the function of a mutant gene. This problem can be addressed by combining multiple mutations or using dominant negative mutants. Finally, conditional gene targeting is needed to determine whether transcription factors required for the development of two or more lineages function in multipotent progenitors and/or lineage-committed progenitors. Understanding the hierarchy and molecular functions of specific hematopoietic transcription factors will lead to a more sophisticated understanding of blood cell malignancies

involving mutations in these genes and will allow new forms of gene therapy utilizing HSC or multipotent progenitors to treat hematological or immune system disorders.

Role of the Microenvironment in HSC Differentiation

The microenvironment in which HSC multiply and differentiate plays a critical role in immune system development. Bone marrow is the major site of HSC development in the adult, and bone marrow transplantation has become a feasible clinical treatment to restore dysfunctional or ablated immune systems. However, whole bone marrow also contains mature T cells that can provoke graft-*versus*-host disease (GVHD). In some clinical protocols, T cells are removed before transfer, but this approach is not always successful. To address the problem further, considerable progress has been made in purifying HSC and expanding them in culture. The recovered HSC have immediate use for transplantation and can also serve in the future as vehicles for gene therapy, but limitations remain in the degree to which HSC self-renewal potential can be maintained *in vitro*. Manipulation and transplantation of HSC would be facilitated by their harvest from peripheral blood, and considerable success has been achieved in learning how to mobilize HSC into the bloodstream. Currently, we have only limited understanding of the mechanisms that normally position HSC and their progeny in the marrow, of what controls the release of newly formed blood cells into the circulation, and of optimal methods for engraftment of transplanted HSC in the recipient.

Complete success will require a thorough description of molecules that comprise the relevant bone marrow microenvironment. HSC and their progeny are highly specialized cells that express receptors for many growth and differentiation factors. Although there has been impressive success in identifying such factors, there is reason to believe that more will be discovered. Most of the known factors are stimulators, but there is a growing appreciation that negative regulators could also be extremely important. For example, substances that limit differentiation might prolong the self-renewal potential of HSC, or agents that prevent entry into the cell cycle might confer protection for stem cells during chemotherapy. The recent discovery of a chemokine called SDF-1, PBSF, or CXC4 illustrates how basic research can yield unexpected but nonetheless important information, even in unrelated areas. SDF-1 was discovered on the basis of its expression by marrow stromal cells and its ability to stimulate pre-B cell growth. Mutation of the SDF-1 gene was found to abrogate B lymphocyte formation and also cause postnatal heart defects. Remarkably, the SDF-1 receptor, called fusin or CXC4R, was then found to serve as a coreceptor for human immunodeficiency virus (HIV) infection.

Most of the regulatory molecules used for blood cell formation within bone marrow also have roles in other tissues. For example, the VLA-4/VCAM-1 pair of cell adhesion molecules is essential for implantation of early embryos, as well as for heart development, blood cell formation, and extravasation of cells into sites of inflammation. Antibodies to these molecules can either mobilize HSC into the circulation or block their engraftment in trans-

plant recipients. Many other cell adhesion molecules and ligands are known to be present in the marrow environment, but their roles are less clear. For example, extracellular matrix constituents such as fibronectin, collagen, hyaluronan, and proteoglycans might contribute to structural integrity in marrow, control cell migration, deliver anti-apoptotic signals, immobilize cytokines near responding blood cell precursors, or serve as coreceptors for growth factors. Recent studies in rodents indicate that sex steroids and other hormones can markedly influence the rate of lymphocyte formation in bone marrow. B cell precursors decline dramatically in pregnant or estrogen-treated mice and are elevated in animals that cannot make or respond to estrogens. It is important to learn whether the same relationships are true in humans, because sex steroids promote the maintenance of bone density (forming the basis for estrogen treatment of postmenopausal women), related hormones are exploited for birth control, and estrogen antagonists that are widely used in breast cancer patients are now being evaluated for prevention of the disease in normal women.

Lymphocyte Development and Repertoire Generation

B Cell Repertoire

Once they differentiate from common lymphoid progenitors, B cell progenitors must undergo additional maturation steps through the pre-B and mature B cell stages. Furthermore, despite considerable new knowledge of the molecules and signaling pathways that direct the activation of mature B lymphocytes, little is known of how these events select particular antigen-specific B cells in immune responses. Recent work has shown an important link between components of the innate complement system, such as C3b, and specific B cell activation. This area offers many opportunities for further investigation and will undoubtedly have important consequences for clinical immunology.

Traditionally, studies of B cell development end with the expression of surface immunoglobulin (Ig), which is the antigen receptor on mature B cells. However, it is now known that both antigen-dependent and antigen-independent processes continue to shape the B cell compartment well after Ig expression. For example, recent work has demonstrated that Ig light chain gene rearrangements continue after initial surface Ig expression. This process of secondary VJ rearrangements, called receptor editing, appears to be important in establishing the primary B cell repertoire. It has been proposed to occur in response to avid self-reactivity, although continued rearrangement in the absence of a positive selection step is an alternative explanation. It is known that the Rag-1 and Rag-2 genes that participate in initial Ig gene rearrangements in pre-B cells are reactivated in mature B cells during an antigen response, suggesting that antigen-dependent expression of novel receptors might occur.

The specificity of Ig on mature B cells can also be modified by V(D)J hypermutation. Such mutations within the variable regions permit the selection by antigen of more efficient, higher affinity B cells, and this process is thought to occur in specialized compartments within peripheral lymphoid organs called germinal centers.

Germinal centers are areas of intense cellular activity soon after antigen exposure, and they are also the normal site of isotype switching and secondary rearrangements. Interestingly, recent studies in LTα and TNFR gene knockout mice demonstrated that these cytokines are important for the normal formation of germinal centers. Because hypermutation and affinity selection can still occur in their absence, additional anatomical sites for these important steps of B cell development must exist. Understanding the mechanism for V(D)J hypermutation remains an overarching goal in B cell research, and recent studies have eliminated some of the previously favored hypotheses. Work has now focused, in part, on the role of general mechanisms of DNA replication/repair, because the normal spectrum of V(D)J mutations requires normal function of transcriptionally coupled DNA repair machinery.

Two late stages of B cell development are the generation of persisting B cells of the memory and antibody-secreting phenotypes. Memory B cells express affinity-selected Ig and are critical for immune protection against reinfection, but they are not well characterized. B cells are known to acquire their memory state during or just after transit through germinal centers. The stimuli that induce memory *versus* antibody secretion *versus* cell death are unknown, although certain cytokines and costimulatory interactions between T and B cells, and between B and follicular dendritic cells, appear to be necessary. The second compartment of persistent B cells is composed of the bone marrow plasmacytes, or antibody-secreting cells. These cells remain in the bone marrow for years after immunization and continuously secrete protective amounts of high-affinity antibody. Despite their central importance for immune protection, these cells have not been well characterized.

In summary, the development of B cells continues at the mature B cell stage, and the processes of secondary Ig gene rearrangements and hypermutation produce B cell receptors with increased affinity to allow responses to even small concentrations of antigen. Persistent memory B cells and plasmacytes play critical roles in ensuring protection against a variety of pathogens following vaccination or nonlethal infection, and increased understanding of these later stages of B cell development should allow the production of more effective vaccines.

T Cell Repertoire

HSC-derived progenitor cells differentiate into functional T cells both in the thymus and in other locations that are less well studied. As many as half of the total systemic T cells reside within the gastrointestinal tract, and only a minority of these cells mature in the thymus. Thus, HSC-derived T cell progenitors produce a subset of cells that populate the gut and mature in this context. In the thymus, other progenitors ultimately produce αβTCR (T cell antigen receptor) CD4 helper cells, αβTCR CD8 cytotoxic precursor cells, and cells bearing the γδTCR, whose function is still unclear (also see Chapter 1). Thymic development of the αβTCR T cells has been intensely studied, and major advances in understanding have occurred over the past few years through the use of mouse transgenic and gene knockout technology.

It is known that immature T cells, or thymocytes, first rearrange their TCR β chains, which are then expressed on the surface together with a nonpolymorphic protein to create a pre-TCR. The pre-TCR then mediates signals to inhibit further β gene rearrangement, to begin α chain rearrangement, and to replace the pre-TCR with the unique αβTCR. The smaller subset of γδTCR-positive cells arises after γ and δ gene rearrangement and surface protein expression without traversing a pre-TCR stage. αβTCR-positive cells then test the affinity with which their receptors react with self-antigen:MHC ligands on antigen-presenting cells in the thymus. Reactions that are too strong or too weak lead to thymocyte death. Reactions that are intermediate in strength allow further thymocyte maturation and development into T cells that exit the thymus and can respond to foreign antigens in peripheral tissues. Thus, the functional T cell repertoire is shaped by both positive and negative selection events in the thymus.

Many developmental questions remain partially or completely unresolved. For example, the way in which non-TCR molecules affect positive and negative selection is not known. It is likely that the CD4 and CD8 proteins, which are coreceptors for the TCR, contribute to repertoire selection by direct signal transduction during self-antigen recognition, but they might also affect the conformation of the TCR:ligand complex to enhance TCR signaling indirectly. At the selecting stage, thymocytes are positive for both CD4 and CD8, but after positive selection an individual cell generally expresses only CD4 or CD8, not both, and the cells acquire the potential for specific effector functions that are generally limited to either the CD4 or CD8 subset. The molecular events that determine these different outcomes are not well understood. An interesting role for the differentiation protein called Notch has been described recently, and it is thought that the level of Notch binding to its complementary ligand on neighboring cells serves to titrate some unknown signal, which then directs the fate of that particular thymocyte. Another area that is not well understood is the regulation of thymocyte cell numbers and transit times through the thymus, and the importance of specific thymic stromal elements in the dynamics of thymocyte development. Continued work in these areas is of central importance for understanding immune competence and for developing approaches to satisfactorily reconstitute the immune system after bone marrow ablation or HIV infection.

Natural Killer (NK) Cell Repertoire

NK cells are a specialized lymphoid lineage that differs from the T and B cell lineages by the absence of highly diverse antigen receptors. Nonetheless, NK cells play important direct roles in immune defense against pathogens, they are crucial for rapid antiviral responses, and they modify T and B cell responses indirectly by secreting cytokines such as IFNγ when activated. NK cells are part of the innate immune response to intracellular bacteria, parasites, and viruses, and they are of clinical significance in bone marrow transplantation because they can mediate the rejection of allogeneic bone marrow grafts. Recent studies indicate that NK cells are most closely related to T cells and both these cell types differentiate from a common T/NK cell progenitor

derived from HSC. However, NK cells do not rearrange or express the TCR genes. Currently, little is known about the receptors responsible for NK cell activation, although recent work has clearly shown that NK cells preferentially kill target cells that have lost expression of MHC class I molecules. Furthermore, this effect is due to the expression of relatively nonpolymorphic families of NK cell receptors that bind to MHC I on other cells and actually inhibit NK cell-mediated cytotoxicity. The mouse and human genes encoding these killer inhibitory receptors (KIR) have been cloned. KIRs have been shown to recognize polymorphic MHC I ligands, and they are expressed on overlapping subsets of NK cells. These observations raise important developmental questions: for example, how do NK cells acquire an appropriate repertoire of KIRs to avoid autoimmunity? Some form of selection must occur, perhaps less sophisticated than in T cells, to account for the fact that NK cells are not autoreactive.

Much remains to be learned about the role NK cells play in immune responses *in vivo*. Clues derived from a few human immunodeficiency patients who lack NK cells but have a normal complement of T and B cells confirm the important function of NK cells in antiviral immunity. Unfortunately, experimental animals that mimic these human patients are not yet available. A greater understanding of normal NK physiology would be advanced by the identification of genes that are uniquely required for NK cell development, thus permitting gene targeting to generate NK knockout mice for basic studies.

Signal Transduction and Lymphocyte Development

B Cells

B cell development from HSC is characterized by a series of checkpoints that progenitor cells must pass through on the road to maturation. Developing B lymphocytes that lack an appropriate characteristic, such as Ig light chain expression, become blocked at a specific checkpoint until they attain that characteristic or they die. Clearly, understanding the nature of these checkpoints requires identifying the signaling events that are needed to satisfy the checkpoints as well as the elements that trigger these signals. The best understood checkpoint in B cell development is the step at which the pre-BCR (pre-B cell antigen receptor; composed of a rearranged Ig heavy chain and two nonpolymorphic surrogate light chains) is expressed on the surface and provides signals for further maturation. Gene knockout mice that cannot express the pre-BCR are severely inhibited in this critical B cell maturation step. Mutation of the intracellular protein tyrosine kinase, Syk, also blocks this step, confirming that Syk plays an essential role in pre-B cell signaling. It is not yet known how pre-BCR signaling is stimulated, but many investigators favor the hypothesis that stromal cells express a generic ligand for the pre-BCR that triggers its signaling function. The identity of this ligand is not yet known but is the subject of intense current investigation.

One limitation of the gene knockout approach is that it may not be possible to assess the impact of the mutation on the process in question if earlier events in development are also dependent on the gene of interest. For example, if the gene

is needed for embryonic survival or for earlier stages of hematopoiesis, the effects on B cell maturation cannot be determined. Recently, two technological advances were developed to overcome this problem in certain circumstances: (1) Rag-2-deficient blastocyst complementation, which allows introduction of the gene mutation only into the lymphoid system (see Chapter 1), and (2) conditional gene ablation using the Cre-loxP system that allows mutation of the gene only in specific cells (see Chapter 15). A third promising strategy is to express an interfering mutant form of the gene (a dominant negative form) *via* a transgene expressed only in B lineage cells. The latter approach has been used recently to express a dominant negative form of the signal transduction component, Ras, in developing B cells. Unexpectedly, dominant negative Ras expression interfered with a step in B cell development prior to the pre-BCR checkpoint, thus opening a new area for investigation. Similarly, deletion of Ig-β, which is needed for pre-BCR assembly and signaling, also led to a block in B cell development earlier than expected. Thus, it is likely that the general approach of interfering with signal components by genetic manipulation in mice will reveal new checkpoints in B cell development.

The practical results of such basic discoveries should benefit clinical approaches for B cell reconstitution, the treatment of various B cell leukemias, and the identification of specific lesions in immunodeficiency diseases, such as the recent identification of ZAP70 and Jak3 mutations as the basis for certain forms of human severe combined immunodeficiency. Genetic manipulation of mice coupled with other experimental approaches, together with basic advances in understanding the human *versus* mouse immune systems, will allow significant progress over the next few years. *In vitro* biochemical studies and *in vivo* experiments with transfected cell lines have already led to a partial understanding of the function of the protein tyrosine kinase gene, Btk, which is defective in the most prevalent human B cell immunodeficiency, Bruton's X-linked agammaglobulinemia (see Chapter 3).

T Cells

Maintenance of T cell homeostasis demands that the daily production of mature T cells conform to a narrow specification. Furthermore, T cell subsets, defined by function as well as physical location, must be appropriately balanced. To achieve satisfactory regulation of T lymphopoiesis, immature T cell progenitors respond to soluble factors and stromally derived cues. For example, immature thymocytes sense the assembly of a satisfactory rearranged TCR β chain gene by signals received through the pre-TCR to promote further cellular maturation. This process provides a useful paradigm for all aspects of T cell development, which requires a series of closely choreographed receptor-derived signals. It is known that pre-TCR signals activate the protein tyrosine kinase, Lck, which then initiates a program to regulate both replication and gene rearrangements. Detailed descriptions of these molecular events will emerge over the next few years as additional information accrues in cell biology and gene mapping and sequencing. Cell fate determination is thought to depend on external signals that control

the ratios of T cell subsets that emerge from the thymus. For example, most mammals produce αβTCR T cell repertoires in which CD4 cells outnumber CD8 cells by nearly 2:1. Recent experiments indicate that the Notch protein may play a role in determining this ratio, as well as in the relative representation of γδ T cells.

The T cells that emerge from the thymus and other sites of maturation undergo further development in a variety of peripheral locations: in the spleen and lymph nodes following antigen exposure and presumably within the respiratory, urogenital, and gastrointestinal tracts. One well-described feature of peripheral development, and the object of intense current study, is the differentiation of mature CD4 T cells into even more specialized cells that can produce only a subset of cytokines. These have been called type 1, or Th1, cells if they produce IL-2 and IFNγ, and type 2, or Th2, cells if they produce IL-4 and IL-10 (also see Chapter 4). Type 1 CD4 cells that direct the maturation of cytotoxic CD8 T cell precursors in response to antigen usually predominate. Under certain circumstances, however, activated CD4 cells are biased toward production of type 2 cytokines that promote vigorous antibody secretion by B cells. This late differentiation step is known to be directed in part by local cytokines, and it provides an obvious target for clinicians interested in molding specific immune responses to attenuate allergic, inflammatory, or autoimmune responses; to control viral infections; or to develop more effective vaccines.

Another major area of current interest is the generation and maintenance of the memory T cell compartment. Although memory T cells are critical for immune protection, they are poorly characterized in terms of the signals that induce long-lived memory *versus* apoptotic death following antigen activation. The role of antigen persistence in maintaining memory T cells is also not well understood, and studies are needed to determine the ability of memory cells to further differentiate *in vivo* to produce a different set of cytokines or acquire new functions such as cytotoxicity. T cell memory is a technically challenging area for study, but its central importance in mediating protection following immunization dictates a call for vigorous research in this area (also see Chapter 4).

An additional important but poorly understood feature of the immune system is the control of T cell numbers in the blood, spleen, and lymph nodes. T cell depletion is usually corrected rapidly in the periphery, suggesting that specific mechanisms exist to maintain peripheral T cell numbers within a prescribed range. Recent studies indicate that peripheral T cells become activated and undergo spontaneous proliferation in the absence of IL-2 activity, although IL-2 is a known growth factor for T cells. This paradoxical result occurs in mice lacking an IL-2 receptor subunit or lacking the Jak3 kinase, which is a pivotal mediator of IL-2 receptor signaling. Other evidence supports the idea that, in addition to its growth-promoting activity, IL-2 is involved in T cell apoptosis following activation. Thus, IL-2 acts to restrain T cell proliferation following activation and also plays a role in expanding T cell num-

bers following T cell depletion. The molecular details of these activities are not yet known, and further work is needed to develop therapeutic strategies to augment or reduce T cell representation in infectious or inflammatory diseases. Such information is also relevant for HSC transplantation and T cell reconstitution following elimination of HIV.

A greater understanding of the signal transduction pathways used in normal T cell development will result from study of transmembrane signaling pathways in other systems, with a focus on the protein tyrosine kinases and phosphatases, serine/threonine kinases, and the GRP-binding proteins that regulate proliferation, cell morphology, and protein secretion. These control circuits function ubiquitously and regulate similar behaviors in most cell types. A particular focus for the next few years will be the cystinyl protease (caspase) family of signal transducers. These enzymes, of which 10 are currently known, act to control apoptosis, linking the tumor necrosis factor, Fas, and other receptors to activation-induced cell death. Apoptosis as a means to eliminate unwanted cells is of great importance in the thymus, where most thymocytes undergoing repertoire selection fail to mature, and also in the periphery, to limit expansion of reactive T cells following an immune response. A detailed molecular understanding of caspase regulation could permit the development of agents that would ablate autoimmune responses or amplify cytotoxic responses toward viruses that illegitimately provoke apoptosis.

Research Opportunities

Hematopoietic Stem Cell Differentiation

- Analyze developmental defects induced by mutations of transcription factors to identify genes that are physiologically relevant for hematopoiesis
- Determine the order of transcription factor function in the hematopoietic hierarchy and determine how different cell type-specific transcription factors interact
- Identify intrinsic and extrinsic signals and their associated signal transduction pathways that activate expression or function of cell type-specific transcription factors
- Identify and characterize stromal elements required for normal HSC growth and differentiation into lymphoid and antigen-presenting cell lineages

Use of HSC in Human Disease

- Develop HSC transplant protocols that ensure engraftment and immune reconstitution without GVHD for application in AIDS and other immunological diseases
- Continue studies on the effectiveness of HSC transplantation in treatments for autoimmune diseases
- Develop gene therapy protocols that allow efficient use of HSC or progenitor cells as vehicles to restore or modulate immune responses in humans

Lymphocyte Repertoire Development

- Identify the regulatory factors that control secondary V(D)J rearrangements (receptor editing) in B cells and their relationships to autoimmunity
- Clarify mechanisms of germinal center reactions: Ig somatic hypermutation, affinity maturation, isotype switching, and the generation and maintenance of B cell memory
- Characterize bone marrow plasmacytes responsible for the persistent production of protective antibodies
- Define the molecular processes that regulate CD4 and CD8 expression during T cell maturation and that control gene expression patterns that determine the ultimate effector functions of positively selected CD4 and CD8 T cells
- Expand studies on fate-determining molecules, such as Notch, to define specific interactions and signals that regulate T cell subset determination
- Investigate the regulation of thymocyte homeostasis and the interplay among bone marrow, thymus, and peripheral lymphoid compartments; define the factors that regulate trafficking from the bone marrow to the thymus
- Characterize the processes responsible for nonthymic T cell development
- Define the molecular events that direct NK *versus* T cell lineage development
- Characterize the selection events that determine patterns of inhibitory and stimulatory receptor expression on NK cells
- Develop animal models of impaired NK cell development in the context of an otherwise normal immune system
- Analyze the effects of sex steroids on human lymphocyte development

Signals That Control Developmental Checkpoints

- Characterize signal transduction requirements at defined checkpoints in B, T, and NK cell development
- Define the parameters that control development of the microenvironments in bone marrow and lymphoid organs required for normal lymphocyte development
- Develop tissue-specific, inducible gene knockout and transgene strategies for ***in vivo*** analysis of developmental signals
- Define specific signal transduction pathways and transcription factor expression required for early and late B, T, and NK cell differentiation
- Develop ***in vivo*** strategies to divert cytokine responses and impose dominant regulation by one ***versus*** other T cell subsets (e.g., CTL, Th1, Th2)
- Characterize the signals that control peripheral lymphocyte homeostasis
- Identify signals that control memory cell development ***versus*** apoptosis following T cell activation

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Primary Immunodeficiency Diseases

3

Overview

Because the immune system is complex and vital to human well-being, defects in one or more of its components occur frequently and can produce a wide spectrum of serious illnesses. In aggregate, these immune system disorders affect large numbers of people and can be either genetically determined (primary) or acquired (secondary). Although the exact incidence of these diseases is unknown, it is estimated that more than 1 in 500 U.S. citizens are born with some type of immune system defect. Many more individuals will acquire a transient or permanent immunodeficiency that may have devastating consequences.

The more than 70 primary immunodeficiency diseases described to date are inherited defects of the immune system characterized by frequent or unusually severe infections. Only mutations of those genes that are important for the function of T or B cells, phagocytes, natural killer cells, or complement will cause primary immunodeficiency disorders. Although some inherited defects may not become apparent until later in life, it is estimated that there are 5,000 to 10,000 infants and young children with severe, life-threatening forms of primary immunodeficiency. The most dramatic inherited disease of the immune system is severe combined immunodeficiency (SCID), which is responsible for the "boy in the bubble" syndrome. Infants who have SCID are born without an adequately functioning immune system and, without early diagnosis and immune reconstitution, they die from infection before their first birthday. Because long-term and/or intensive treatments are required, the total medical, economic, and emotional impact on society of primary immunodeficiency diseases is enormous.

Secondary immunodeficiency can result from other diseases or from the therapies used to treat them. The emergence of AIDS in the early 1980s, caused by infection of immune cells with the human immunodeficiency virus (HIV), resulted in markedly increased awareness of this type of problem. HIV infection cripples the immune system and leaves the afflicted individual defenseless against pathogens. Other acquired immunodeficiencies result from various types of infections, drug therapies, exposure to radiation, or malnutrition.

Clearly, immunodeficiency diseases can be devastating, but they have provided scientists the opportunity to understand many important aspects of the human immune system. Studies of affected patients have already identified crucial molecules, pathways, and structures that play a major role in the defense against microorganisms, prevention of tumors, and protection from autoimmune diseases. Understanding these mechanisms has also resulted in the development of new approaches to regulate the immune system with synthetic substitutes for, or antibodies to, substances normally

Table 3-1. Abnormal Genes Known To Cause Human Primary Immunodeficiency*

Chromosome	Gene Product	Disorder
1q	RFX5†	MHC class II antigen deficiency
1q25	$p67^{phox}$†	Autosomal recessive CGD
1q42-43	vesicle membrane component†	Chediak-Higashi syndrome
2p11	Kappa chain†	Kappa chain deficiency
2q12	ZAP70†	CD8 lymphocytopenia
5p13	IL-7Rα†	T-B+NK+SCID
6p21.3	TAP†	MHC class I antigen deficiency
6p21.3	Unknown	IgA deficiency; CVID
6q22-q23	IFNγR1†	Disseminated mycobacterial infections
7q11.23	$p47^{phox}$†	Autosomal recessive CGD
8q21	Unknown	Nijmegen breakage syndrome
9p21-p13	Unknown	Cartilage hair hypoplasia
10p13	Unknown	DiGeorge/velocardiofacial syndrome
10p14-15	IL-2Rα†	Lymphoproliferative syndrome
11	CD3 γ† or ε†	CD3 deficiency
11p13	RAG1 or RAG2†	T-B-NK+SCID
11q22.3	DNA-dependent kinase†	Ataxia telangiectasia
13q	RFXAP†	MHC class II antigen deficiency
14q13.1	Purine nucleosidase†	PNP deficiency
14q32.3	Immunoglobulin heavy chains†	B cell negative agammaglobulinemia (μ) or selective deficiencies of other isotypes
15q21	myosin-Vα†	Griscelli's disease (partial albinism)
16p13	CIITA†	MHC class II antigen deficiency
16q24	$p22^{phox}$†	Autosomal recessive CGD
19p13.1	JAK3†	T-B+NK-SCID
20q13.11	ADA†	T-B-NK-SCID
21q22.3	CD18†	Leukocyte adhesion deficiency, type 1 (LAD1)
22q11.2	Unknown	DiGeorge/velocardiofacial syndrome
Xp21.1	$p91^{phox}$†	X-linked CGD
Xp11.23	WASP†	Wiskott-Aldrich syndrome
Xp11.3-p21.1	Properdin†	Properdin deficiency
Xq13.1	Common γ chain (γc)†	T-B+NK-SCID
Xq22	Bruton tyrosine kinase (Btk)†	X-linked (Bruton's) agammaglobulinemia
Xq24-26	Unknown	X-linked lymphoproliferative syndrome
Xq26	CD154 (CD40 ligand)†	X-linked hyper-IgM syndrome

†Gene cloned and sequenced, gene product known.

*Modified from: Kokron, C.M.; Bonilla, F.A.; Oettgen, H.C.; Ramesh, N.; Geha, R.S.; Pandolfi, F. Searching for genes involved in the pathogenesis of primary immunodeficiency diseases: Lessons from mouse knockouts. *J. Clin. Immunol.* 17:109–126, 1997, with permission.

produced by the body, such as IFNγ, G-CSF, IL-2, CD40L, CTLA4, and TNFα. Immunodeficiency diseases may involve either the innate or the adaptive immune systems. Defects of phagocytic white blood cells or of serum complement components are examples of disorders that affect nonspecific innate immunity, whereas defects of specific adaptive immunity might involve altered development or function of either T or B cells. Until the past few years, there was little insight into the fundamental problems underlying most of these conditions, but there has been a recent explosion of information leading to the identification of genes responsible for many classic immunodeficiencies (Table 3-1). These remarkable advances have been made possible through new knowledge in molecular and cellular immunology. One specific example is the current ability to develop gene knockout mice with deletions of specific components of the immune system. The biologic consequences of such mutations can provide clues to molecular causes of similar problems in humans. A list of gene-targeted mice that lack specific components of the immune system and the human chromosomes on which the corresponding genes reside is presented in Table 3-2.

Within the past 5 years the molecular bases of four X-linked immunodeficiency disorders were discovered: X-linked agammaglobulinemia, X-linked immunodeficiency with hyper-IgM, the Wiskott-Aldrich syndrome, and X-linked severe combined immunodeficiency (Table 3-1). The abnormal gene in X-linked chronic granulomatous disease (CGD) had been identified several years earlier, and the gene encoding properdin (mutated in properdin deficiency) has also been cloned. The faulty gene in X-linked lymphoproliferative disease has been localized to a specific site on the X chromosome but as of this writing has not yet been identified. Autosomal recessive immunodeficiencies for which the molecular bases have been discovered include leukocyte adhesion deficiency type 1; adenosine deaminase deficiency (ADA); purine nucleoside phosphorylase deficiency; ataxia telangiectasia; DiGeorge syndrome; MHC antigen deficiency; ZAP70 deficiency; Jak3 deficiency, and IFNγ receptor deficiency (Table 3-1). The discovery and cloning of the genes for these diseases have obvious implications for the potential of gene therapy. The rapidity of these advances suggests that there soon will be many more to come. However, the genes identified thus far represent a small percentage of the many defective genes responsible for primary immunodeficiencies.

During the past two decades, major advances have also occurred in the therapy of immunodeficiency diseases. Among the most important advances are (1) the development of several safe and effective forms of intravenous immunoglobulin for treating antibody deficiency; (2) techniques for removing mature T cells from bone marrow cell suspensions, thereby permitting half-matched parental bone marrow stem cell transplantation in patients with SCID or other lethal genetic defects in the hematopoietic system; (3) development of new antibiotics that are effective against opportunistic infectious agents; and (4) recombinant cytokines for treating a variety of immunodeficiencies. As successful as these treatments have been for some of these diseases, treatment

Table 3-2. Human Chromosome Locations of Genes With Immunological Phenotypes in Mouse Mutants*

Human Chromosome	Genes
1	CD2, CD3η, CD3ζ, CD30, CD45, CR2, E-selectin, FasL, FcγRII, FcγRIII, FcRγ, IL-10, L-selectin, Lyst, p56lck
2	CD8α, CD8β, CD28, CTLA-4, IL-1β, IL-8R, **kappa chain,** Ku80, substance P receptor, **ZAP70**
3	B7-1, IL-5Rα
4	IL-2, IRF-2, NF-κB (p50 subunit)
5	CD14, GM-CSF, Granzyme A, IL-4, IL-5, IL-7R, IL-12 p40, IRF-1, itk, Ii (invariant chain), Tcf-1
6	c-myb, **C4,** IFNγ receptor α, LMP-2, LMP-7, MHC class II, p59fyn, Pim-1, SOX-4, TAP1, TNF-β
7	Ikaros, IL-6, TCRβ chain
8	DNA-dependent kinase (p350), DNA polymerase β, IL-7, Lyn
9	abl, Pax5/BSAP, Syk
10	**Fas/CD95, IL-2Rα,** PBSF/SDF-1, Perforin, TDT
11	**ATM, CD3**ε, CD5, ETS-1, NF-κB (p65/Rel-A subunit), **RAG-1, RAG-2**
12	β7 integrin, CD4, GATA-2, IFNγ, Lag3, P-selectin, p27 kip1,TNFR p55(α), STAT-6
13	flk-2
14	c-fos, IgD, IgE, IgM, immunoglobulin Gm-2b, JH, switch γ1, TCRα chain, TCRδ chain
15	β2-microglobulin, p50csk, MYO5a
16	CD19, CD43, PKCβ
18	Bcl-2
19	Bax, C/EBPβ, **C3,** C5aR, CD23, E2A, ICAM-1, **Jak-3,** OCT-2, TGF-β1, Vav
20	**ADA,** CD40, **NFAT1**
21	**CD18**
22	IL-2Rβ, IL-3/GMCSF/IL-5 Rβ, lambda 5
X	**btk, CD40L/CD154, IL-2R**γ

*Modified from: Kokron, C.M.; Bonilla, F.A.; Oettgen, H.C.; Ramesh, N.; Geha, R.S.; Pandolfi, F. Searching for genes involved in the pathogenesis of primary immunodeficiency diseases: Lessons from mouse knockouts. *J. Clin. Immunol.* 17:109–126, 1997, with permission. Bolded defects have known human counterparts.

is far from satisfactory for many others. The promise for even greater accomplishment in treating immunodeficiency diseases lies in the rapidly evolving understanding of the primary causes of these diseases and in the infinite potential of molecular biological approaches to therapy. Continued progress will require the support of research conducted by clinical scientists who care for patients with immunodeficiencies, study of these rare patients at a cellular level, and evaluation of innovative therapies. These efforts must be combined with the work of basic scientists who investigate the molecular aberrations associated with immunodeficiency diseases.

Identification of Faulty Genes and Gene Products

To develop targeted therapies for immunodeficiencies, scientists need to identify the faulty genes and gene products for each of the primary immunodeficiency diseases. This effort will require the coordinated use of family studies, modern molecular biology, and studies of cell function and specific protein products. The strategies currently used to enroll families in cytogenetic and linkage studies will continue to reveal additional human immunodeficiency genes; such studies require cooperative ventures among patients, their families, and the scientists. New advances in molecular genomics, such as polymerase chain reaction-based polymorphic markers; databases of expressed sequences; and statistical approaches to identifying genes contributing to complex disorders should all be incorporated into this effort.

Use of Mouse Mutants To Define Uncharacterized Human Immunodeficiencies

Gene knockout models of primary immunodeficiencies in mice (Table 3-2) have the obvious potential to provide insight into the possible consequences of mutations in the corresponding genes in humans. Although the phenotypes of gene mutation in mice and humans are not always exactly the same, there are analogies in a sufficiently high number of cases to suggest that a thorough knowledge and access to the database of gene knockouts in mice will facilitate the pursuit of novel gene defects as a basis of primary human immunodeficiency.

Functional Defects in Primary Immunodeficiencies

Identification of the genes mutated in primary immunodeficiencies is only the first step. Although we know that many of the proteins encoded by these genes are components of signal transduction pathways that control lymphocyte proliferation, differentiation, or activation, the exact normal functions of many of these proteins and the mechanisms by which defects in these proteins result in immunodeficiency are not yet known. To develop new therapeutic approaches, the molecular roles of these mutated proteins must be elucidated. Such studies have already introduced us to previously unknown critical cellular pathways that now must be dissected and understood. Currently available techniques of monoclonal antibody production, cellular expression constructs, experimental introduction of natural and targeted mutations, and the utilization of

yeast two-hybrid systems for identifying interacting gene products can be applied in these studies.

Occasionally, defects in corresponding genes may have different consequences in humans and mice. For example, defects in the Btk gene cause a severe immunodeficiency in man called X-linked agammaglobulinemia but a much milder defect in mice. By contrast, abnormalities in two genes involved in the response to growth factors result in the absence of both T and B cells in the mouse, whereas humans with defects in the same genes have absent T cells but normal or elevated numbers of B cells. These differences suggest that there is some flexibility in development of the immune system. Learning how to use this flexibility may allow us to enhance the immune system in patients with immunodeficiency and suppress the immune system in autoimmunity.

Study of Immune System Inhibitory Proteins

A number of proteins that serve as negative regulators of the immune system have been discovered in recent years, and it is clear that deficiencies of these components of the immune system might represent another form of immunodeficiency. For example, the cell surface receptor Fas is an important mediator of programmed cell death (apoptosis), which is crucial to the termination of physiologic immune responses. Defective expression of Fas in the human autoimmune lymphoproliferative syndrome (ALPS) leads to the accumulation of lymphocytes in lymph nodes and blood and to autoimmunity. Recently, deficiencies of three other proteins, the α or β chains of the T cell growth factor (IL-2) receptor or the T cell molecule CTLA4, have led to a similar picture of lymphoproliferation and autoimmunity in mutant mice and in a human patient with a defective IL-2 receptor α chain. In addition, there are negative regulatory molecules on natural killer (NK) cells called killer inhibitory receptors, or KIRs, that interact with self-MHC class I molecules to send a message to the NK cells not to kill autologous cells. If these receptors are occupied by viruses or are mutated, it is possible that this negative control would be lost. It is likely that there are other negative regulators of the immune response yet to be discovered, since the clinical picture present in ALPS also has been seen in patients who do not have deficiencies in Fas.

Therapeutic Approaches

Animal Models of Gene Therapy

The identification of many of the genes responsible for immunodeficiency has created a sense of enthusiasm about the possibility of gene therapy, and scientists have begun to design therapies using normal genes to replace defective ones. However, a variety of problems must be solved before gene therapy is feasible. Research now must focus on methods for stable integration of the normal gene with high efficiency into the DNA of a self-renewing cell population, preferably a precursor cell pool. Basic research on the biology of stem cells and mechanisms of gene transfer will address these problems. Scientists then must determine how to transcribe and translate the gene to generate adequate but nontoxic concentrations of the gene product. Gene correction by homologous recombination is one

approach with great potential value, because expression would be regulated in a completely physiologic manner. Animals deficient in the same genes that cause immunodeficiencies in humans can be used to determine whether small amounts of the corrected protein are clinically beneficial or whether high concentrations of the protein product have unacceptable side effects.

Replacement Therapy

In addition to continuing efforts toward gene therapy, researchers must improve current therapies that replace missing enzymes, antibodies, and cytokines by chronic treatment with modified natural proteins or recombinant molecules, some of which must be taken up by cells to be functional. With the exception of missing blood serum antibodies, few clinical immunodeficiencies are now being treated successfully by replacement therapy.

Bone Marrow Transplantation

Bone marrow transplantation is currently the most effective permanent therapy for primary immunodeficiency diseases. Marrow transplants have proven capable of mitigating the profound immunodeficiency of human SCID, and infants with SCID who receive T cell-depleted haploidentical (half-matched) parental stem cell transplants provide a valuable opportunity to study development of the human immune system. However, depending on the genetic cause of the SCID, the immune reconstitution may not be complete. Moreover, since this type of transplant has been possible only for the past 16 years, it is too soon to know whether immunoreconstitution is truly permanent. Since this is still the most effective type of therapy for SCID, it will be important to determine the extent of development and the degree of persistence of immunity in these patients.

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Research Opportunities

Genetic and Functional Defects in Human Primary Immunodeficiency Diseases

- Identify as yet unrecognized or poorly defined human primary immunodeficiency diseases; study relevant gene knockout mice to discover novel causes of human immunodeficiency
- Identify the functions of newly identified immunodeficiency-causing gene products in both mice and humans
- Increase knowledge of the similarities and differences between the human and mouse immune systems
- Identify and characterize genes involved in complex and/or rare immunodeficiencies for which the molecular basis is not known, using a combination of family genetic studies, studies of cell function, modern molecular biology, and biochemical studies of specific gene protein products
- Identify deficiencies of known or unknown negative regulators of the immune system in patients with lymphoproliferative and/or autoimmune diseases
- Study patients with SCID who have received haploidentical T cell-depleted bone marrow stem cell transplants without pretransplant chemotherapeutic conditioning as models for normal development of the human immune system

Therapies for Primary Immundeficiency Diseases

- Develop gene therapies for primary immunodeficiencies; use animal models to help evaluate the expression and regulation of the transferred genes
- Improve replacement therapies with enzymes, antibodies, or cytokines
- Develop new therapies: explore *in utero* bone marrow transplantation; unrelated, partially HLA-mismatched cord blood transplants; cytokine therapy; and thymic epithelial transplants

Infrastructures To Facilitate Research, Diagnosis, and Treatment

- Establish repositories of human cell lines from immunodeficient patients and repositories of frozen embryos from relevant animal models
- Establish Internet communication to share information among basic and clinical investigators
- Apply knowledge gained about genetic causes of disease to genetic counseling and prenatal or perinatal diagnosis so that early therapy can be implemented

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Immunoregulation

4

Overview

The immune system was first appreciated for its role in defense against infections. Advances in understanding immune cells, their functions in antimicrobial protection, and mechanisms for their regulation were achieved during the late 1970s and 1980s through studies that separated and/or cloned the cellular and soluble components using tissue culture systems. Building on this information, the past decade has provided extensive data concerning regulation of antimicrobial immune defenses within the infected host. This work resulted in a number of surprises and has clearly demonstrated the importance of examining immunoregulation within the environment of complex endogenous responses. These lessons extend to the control of other immune responses, such as those in allergy, autoimmunity, tumor immunology, and allotransplantation. Although great progress has been made, much remains to be learned about regulation of the complex pathogen:immune system interactions that occur during microbial challenges. Breakthroughs in disease therapy based on immunotherapeutic approaches will depend on a thorough understanding of both the conditions required to promote antimicrobial states and the positive and negative consequences of modifying immune responses in infected individuals or those with immune-mediated disease.

Regulation of Effector Functions

With many microorganisms, initial infection leads to a rapid inflammatory response accompanied by the production of cytokines such as IL-1, IL-6, and TNF by monocytes and fibroblasts, as well as IL-12 and IGIF (IL-18) by macrophages. Activation of this initial innate immune response can influence the subsequent antigen-specific T and B cell responses elicited to a particular pathogen. Undoubtedly, chemokines and their receptors also play an important role. The level and class of the immune response generally determine whether the pathogen will be eliminated. Both inflammatory and antigen-specific responses are tightly regulated in order to avoid immunopathology such as septicemia or shock that might result from excess production of these soluble mediators. The cytokines IL-10 and TGFβ play an important role in downregulation, and a role for the T cell surface molecule, CTLA-4, has been shown in the regulation of antigen-specific T cells.

Effector Classes

It is now clear that different types of immune effector functions can be specifically activated and tightly regulated after infection. Although a number of mechanisms can overlap in contributing to defense, certain cellular, cytokine, and antibody responses are particularly important against the different types of infectious agents: bacteria, parasites,

fungi, and viruses. For example, macrophages mediate the most important defense against intracellular bacteria and parasites; antibodies defend against extracellular parasites; and cytokines, natural killer (NK) cells, and CD8 T cells all mediate important defenses against viral infections (Figure 4-1).

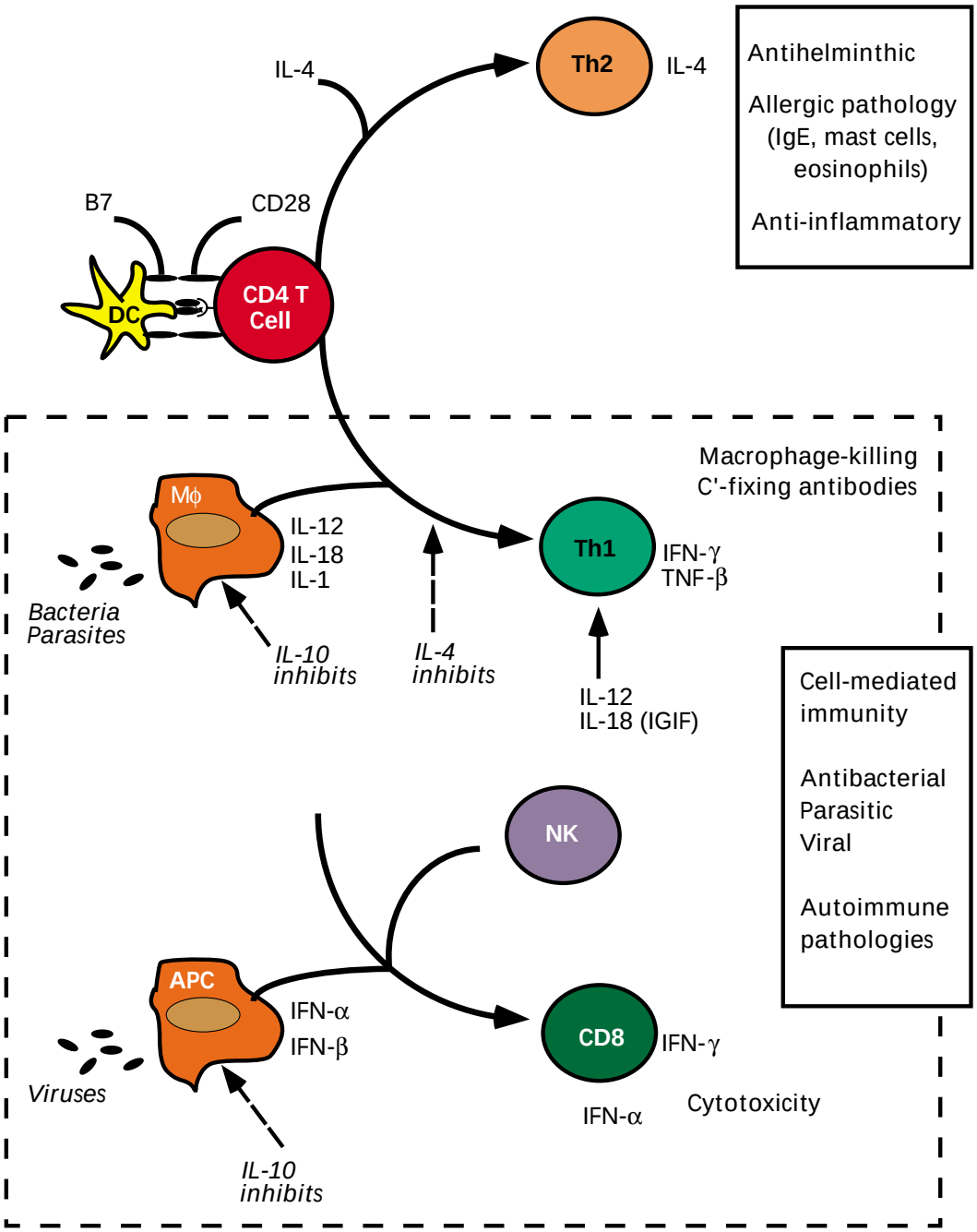

Figure 4-1. Development of Effector Functions During an Immune Response

Source: Anne O'Garra, Ph.D.

Cytokines signal and regulate different types of effector functions. Macrophages are activated by TNF and/or IFNγ for microbial killing functions, and NK cells produce IFNγ early after exposure to IL-12, which is induced by some infectious agents. IL-12, IGIF, and IFNγ then stimulate activated Th1 cells to produce IFNγ. Th2 cells produce IL-4 and IL-5, which promote antibody- and eosinophil-mediated effector responses, respectively. NK cytotoxicity is activated by IFNα and IFNβ, which are produced by virally infected cells. Although the mechanisms are still not well understood, IFNα/β may promote cytotoxic activity and IFNγ production by CD8 T cells. Thus, different effector cell responses are required to fight different infectious agents, and the conditions induced by the infectious agent can promote the particular response needed for protection. In some cases, however, the infectious agent may induce a type of response that allows it to escape immune attack and survive in the host, thus circumventing protective immune mechanisms.

These responses must be tightly regulated because those that optimally induce a subset of effector responses can inhibit other effector functions needed to control other infections. Furthermore, certain cytokines can be detrimental to the host if left unchecked or if inappropriately produced together with other cytokines. It is known that IFNγ can inhibit Th2 responses, and conversely, that IL-4 and IL-10 interfere with Th1 responses. In addition, TGFβ and IL-10 inhibit IL-12 production. There are also a variety of noncytokine factors that help regulate immune responses, such as the prostaglandins and glucocorticoids.

Interestingly, glucocorticoids are induced as products of a neuroendocrine cascade during certain viral infections and under conditions of systemic cytokine responses to sepsis caused by Gram-negative bacteria. The glucocorticoid response can contribute to negative regulation of endogenous cytokine responses and mediate protection against cytokine-induced pathology.

Thus, there are a number of pathways to ensure that responses to particular infectious agents preferentially promote the required immune effector functions, and that these responses are carefully controlled. However, much remains to be learned before predictable modulation of human immunity and disease can be realized. Studies of endogenously produced cytokines and of therapeutic cytokine uses may have critical implications for development of strategies to fight disease. Further pursuit of current research opportunities will result in a thorough characterization of the complex interactions that occur in infected hosts and should permit rational design of immune interventions.

Inflammation

Inflammatory responses constitute an essential but double-edged sword. Inflammation is absolutely necessary to heal our wounds and protect us from invading microorganisms, but can also be the basis of severe and sometimes lethal tissue damage. The past 10 years have produced enormous advances in the knowledge of many inflammatory components and their activities under different conditions (see Chapter 5 for further discussion). A major focus has been the role

played by cytokines and cell adhesion molecules in triggering and regulating inflammation as well as determining the character of inflammatory lesions. The recent realization of the fundamental importance of chemokines and their receptors in governing cellular responses, and the development of knockout animals and other tools for analyzing chemokine functions, should now lead to a very exciting research era in which the detailed events involved in the orchestration of complex tissue reactions can be elucidated. Such information is likely to be critical for the design of intervention strategies that block the pathologic consequences of the inflammatory response without compromising host resistance. In addition, new insights into possible targets for intervention are likely to emerge from studies on the basis of differential cell signaling and neuroendocrine influences on the inflammatory process. Rapid progress can also be expected in the elucidation of previously unappreciated microbial stimulants of inflammatory disease along with the mechanisms by which they influence the development of self-detrimental T cell responses.

Regulation of Lymphocytes

T Cell Subsets

Different effector mechanisms are appropriate for different pathogens. For example, the types of mechanisms efficient for the elimination of an intracellular bacterium are different from those that act against intestinal nematodes, and the immune system is often able to discriminate among such diverse organisms and mount the appropriate response. However, disease can develop because of inappropriate responses. The understanding of how these different types of immune responses are generated following infection was significantly advanced with the discovery that CD4 T cells can be separated into subsets, termed Th1 and Th2, based on which cytokines they produce. Recent work has shown that CD8 cells also form two subsets. Although less information is available on the physiological relevance of the CD8 subsets, both demonstrate cytotoxic activity. Th1 cells are primarily defined by their ability to produce IFNγ and lymphotoxin. Th1 responses are often accompanied by the activation of CD8 T cells as well as the production of complement-fixing antibodies by B cells (Figure 4-1). In contrast, Th2 cells produce IL-4, IL-5, and IL-13, which are implicated in the eradication of some helminthic parasites and are associated with the pathologies that accompany allergic disease. Regulatory CD4 subsets that can inhibit both Th1 and Th2 cells have been demonstrated recently, but they are less well characterized than Th1 and Th2 cells. The cytokine TGFβ has been implicated in the function of such regulatory cells.

Importantly, once a dominant Th subset develops, cytokines produced by that subset inhibit development of the alternative Th cell type. Polarized Th1 and Th2 responses represent endpoints of chronic inflammation or chronic disease. At a single cell level, once a Th1 or Th2 cell has differentiated, it is committed and cannot be induced to change its cytokine profile. However, at a population level *in vivo*, a range of different activation and maturation states of the Th cells will undoubtedly exist during either a “Th1” or “Th2” response. Thus, although a Th1 or Th2 phenotype is irreversible at the single cell

level, it may be reversible at a population level. This concept has important implications for therapeutic intervention. Discovery of the Th1 and Th2 subsets provides a foundation for studies aimed at defining the factors that determine which T cell subset will dominate after an infection or during an autoimmune attack or allograft rejection, and for an analysis of subset regulation. Both issues have important implications for the treatment of disease.

It is very likely that the development of a T cell subset depends on the nature of an invading pathogen, its route and dose of entry, and the genetic background of the host. The most clearly defined factors are the cytokines present at the beginning of the immune response. For example, bacteria stimulate macrophages to produce IL-12 and IGIF, and stimulate NK cells to produce IFNγ. These cytokines then drive the development and maintenance of Th1 responses. Conversely, production of IL-4 early in the response directs naive cells to develop into Th2 cells. The source of the early IL-4 burst and mechanisms for its induction are not yet known. IL-12 directs Th1 development by activating signal transduction proteins called Stat3 and Stat4 (Figure 4-2). Among CD4 T cells, functional IL-12 receptors (IL-12R) appear to be restricted to recently activated, uncommitted cells and to Th1 cells. IL-12R are lost during Th2 differentiation, at least in part because IL-4 downregulates one of the IL-12R subunits.

Th1 development also depends on IFNγ, which can act on macrophages to increase IL-12 production or can act directly on the T cell. Other cofactors also contribute to Th1 development. IGIF synergizes with IL-12 to induce IFNγ secretion from Th1 and NK cells, and IGIF gene knockout mice have a severely impaired ability to produce IFNγ in response to bacterial antigens. Thus, blocking either IL-12 or IGIF signaling may be beneficial in treating Th1-associated autoimmune pathologies. Unlike IL-12, IGIF does not activate Stat4 in Th1 cells, but it does signal through another pathway (IRAK) to induce a gene-regulating factor called NFκB. The cytokine IL-1α has no effect on Th1 cells, but it activates NFκB and induces proliferation in Th2 cells. IL-4 signals T cells by activating Stat6 and inducing phosphorylation of the insulin receptor substrate, IRS-2. Stat6 also plays a role in turning on IL-4 production. Although deletion of the Stat6 gene in mice results in deficient Th2 responses, the mechanism by which Stat6 induces Th2 development remains unclear. To date, six regulatory proteins have been implicated in Th2 differentiation, but their functions have not yet been elucidated.

The most direct benefit of understanding the biological functions of Th subsets may be in the area of vaccines. Immunization has historically been an empirical science, and in spite of the large body of recently accumulated knowledge about mechanisms of immunity, the ability to induce a specific type of immune response remains more art than science. However, the discovery that particular cytokines can direct the differentiation of Th1 *versus* Th2 cells has opened up a new era in rational vaccine development. The discovery that IL-12 can initiate cell-mediated immunity through Th1 cells has led to its experimental use as an

adjuvant in vaccines requiring this type of response. Other molecules that contribute to the generation of T cell responses, such as the costimulatory proteins B7-1 and B7-2, are also being exploited to improve vaccine efficacy.

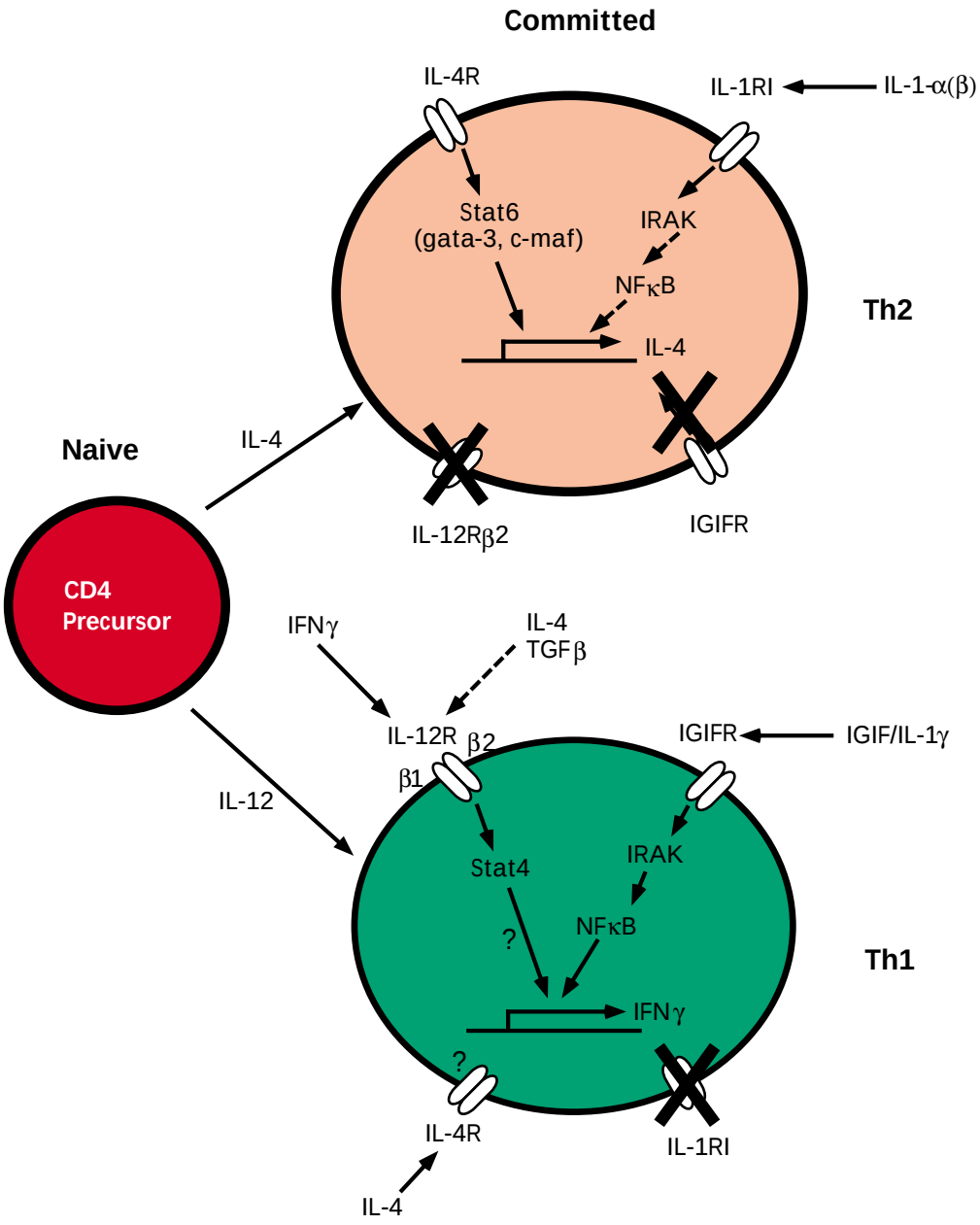

Figure 4-2. Differential Signaling in Th1 and Th2 Cells

Source: O'Garra, A. Cytokines induce the development of functionally heterogeneous T helper cell subsets. *Immunity* 8:275–283, 1998; used with permission; copyright held by Cell Press.

The inappropriate activation and expansion of particular T cell subsets are sometimes associated with disease. This is most evident in infectious diseases, especially parasitic diseases, as well as in autoimmunity, allergy, and oncology. For example, we now know that pathologic allergic responses represent an aberrant Th2 dominance that promotes the production of IgE and activation of eosinophils and mast cells. In contrast, autoimmune diseases are frequently associated with an exaggerated Th1 response that produces tissue-damaging inflammation and cytotoxicity. Such knowledge provides a rational basis for manipulating immunity away from the inappropriate response, by blocking IL-4 or IL-12/IGIF to inhibit Th2 or Th1 development, respectively. However, it may not always be readily apparent which type of T cell is required to control a particular disease. One obvious example is HIV/AIDS. Studies from many laboratories have shown that a variety of effector mechanisms may control HIV *in vitro*, but the relative importance of each mechanism *in vivo* is much more difficult to ascertain. Such information is needed to target a particular T cell subset during either immunization or immunotherapy. Unfortunately, surface marker proteins that distinguish among the subsets have not yet been defined, although new work suggests that such markers may indeed exist. Recent observations indicate that chemokine receptors are differentially expressed on Th1 and Th2 subsets, suggesting possible therapeutic approaches for intervention in allergic or autoimmune diseases. For example, Th2, but not Th1, cells were found to express the CCR3 and CCR4 receptors that bind the chemokines RANTES and TARC, respectively.

B Cell Subsets

Effector B cell subsets are defined by the expression of different constant (C) regions, or isotypes, in their immunoglobulin (Ig) receptor heavy chains. Initially, all B cells have Cμ (IgM) and Cδ (IgD) receptors. Following antigen stimulation and interaction with T cells and cytokines, Cμ is switched, such that a B cell now produces IgG, IgA, or IgE instead of IgM and IgD. The process of isotype switching involves genetic recombination in which the same antigen-binding VDJ region becomes linked to a new constant region (see Chapter 1). Different T cell subsets and cytokines promote the switch to different Ig isotypes. Each Ig isotype has a unique pattern of functional properties that define distinct pathways for removing antigen from the host. Advances and continuing challenges in the field of Ig isotype switch can be divided into three areas: isotype function, cellular regulation of isotype switching, and the molecular biology of the switch process.

Differences in the abilities of Ig isotypes to fix complement, to bind to cellular Fc receptors and trigger or suppress cellular activities, to resist intestinal or intracellular digestion, and to polymerize upon binding antigen have all been well characterized. However, the *in vivo* advantages that are conferred by the expression of a particular isotype have been difficult to demonstrate. Mice and humans deficient in just a single Ig isotype have no or only subtle defects in the control of infectious pathogens. Although individual isotypes were found to be responsible for immune protection against particular pathogens in normal rodents, there is little evidence that this reflects the special properties of

a particular isotype rather than predominant production of that isotype. Experiments with sets of monoclonal antibodies that differ in isotype but have identical VDJ regions have not shown that a particular isotype is essential or even best suited for protection against a particular pathogen. However, it is still possible that particular isotypes are critical for host protection when antibody concentrations and affinity are limiting.

Some differences exist in the way that cytokines regulate isotype switching in mice and humans. Cytokines are known to regulate Ig isotype selection *in vivo* in the mouse, while their *in vivo* roles in human Ig isotype selection are not firmly established. In both species, cytokine effects in cell cultures are influenced by the type of stimulus that induces B cell proliferation and antibody production. For example, IFNγ inhibits switching to IgE by human B cells stimulated with IL-4 and Epstein-Barr virus infection, but not by B cells stimulated by IL-4 and CD40 ligation; and IFNγ stimulates switching to IgG3 when mouse B cells are activated by IL-5 plus membrane Ig cross-linking but inhibits switching to IgG3 when lipopolysaccharide is used to activate the cells.

Understanding of isotype switching at the molecular level has advanced rapidly but remains incomplete. In both mouse and human, switching to a different Ig isotype nearly always involves recombination between two switch (S) region introns on the same DNA strand; one located 5′ to the Ig heavy chain constant (C_H) gene that was expressed prior to switching, and the other located 5′ to the Ig C_H that will be expressed after switching occurs. This results in looping out and deletion of the DNA located between the two switch regions, so that the VDJ segment becomes proximate to the newly expressed C_H gene. S regions contain DNA sequences that may be binding sites for proteins to align DNA into the ordered structure that allows deletional recombination.

S region recombination is always preceded by transcription of germline sequences that include the C_H gene that will be expressed after switching, the S region located 5′ to that C_H gene, and a transcription initiation (I) region that is 5′ to that S region. This ISCH RNA transcript is processed to produce an ICH transcript, which is not translated. Transcription of the germline form of a particular C_H gene is induced by the same stimuli that are associated with switching to that C_H gene. It is debated whether the ICH transcript has an active role in deletional recombination or whether it is the process of transcription across the S region that promotes recombination by making that region accessible to the DNA binding proteins and enzymes that mediate switch recombination; thus, ICH transcripts may be nonfunctional byproducts. Germline C_H gene transcription is not sufficient to induce isotype switching. DNA synthesis is also required, as is an additional stimulus, such as CD40 ligation. This stimulus may be required to produce the enzymes involved in deletional recombination and/or produce DNA binding proteins that direct these enzymes to particular S regions.

Several DNA binding proteins that stimulate germline C_H gene expression and deletional recombination have been identified. For example, Stat6, members of the NFκB/Rel family, and members of the E/EBP family are all involved in stimulating germline γ1 and ε transcription. The NFκB homodimer called SNIP and a complex (SNAP) that contains the transcription factor E74 bind to motifs within the Sγ region and probably promote deletional recombination by producing the double-stranded DNA breakpoints that are required for initiation of switch recombination. By analogy with other DNA recombination processes, it is likely that separate enzymes are responsible for creating double-stranded DNA breaks, for directing the juxtaposition of donor and recipient S regions, and for reannealing DNA in the juxtaposed S regions. It is not known whether a single recombinase enzyme directs all isotype switches or whether switching to different isotypes requires different recombinases.

Regulation of Immunological Memory

One of the most fundamental yet least understood characteristics of protective immunity is the phenomenon of immunological memory. Initial exposure to an antigen by natural means or deliberate vaccination results in the development of antigen-specific memory T and B cells that persist for many years, sometimes throughout life. These memory cells can provide specific and highly effective protection upon antigen reexposure, because they exist in expanded numbers and are more readily activated than naive cells. Furthermore, they may be already biased toward the type of effector response required to protect the host. Although these cells have been difficult to analyze in past years, newly developed approaches are available to facilitate more detailed understanding of memory cell generation, activity, and maintenance.

T Cell Memory

Memory T cells probably derive from activated effector cells. The induction of effector T cells can result in a huge expansion of the specific naive T cells that recognize the antigen. To maintain homeostasis and a diverse T cell repertoire, many of the responding cells must be eliminated after clearing the antigen. The effector phase of a T cell response persists only as long as the antigen can be detected in the body. Following removal of the antigen, most effector cells die by a process of apoptosis called activation-induced cell death (AICD). Some of the mechanisms that control AICD have been described. During antigen stimulation, activation marker proteins become expressed on the T cell surface, including some proteins that trigger cell death. Furthermore, most responding T cells downregulate intracellular proteins of the Bcl family that protect against AICD, rendering them susceptible to death signals in the environment of the waning response. However, a fraction of the responding cells survive to become memory T cells. It is thought that memory cells avoid AICD by maintaining anti-death Bcl protein expression, but the parameters that allow this difference from AICD-susceptible cells are not well defined. It is not even clear whether memory cells and dying effector cells are generated from the same naive precursor cells.

One problem in studying memory T cells is that they are difficult to identify because most of their surface markers revert to a naive status as they stop proliferating and their effector functions cease. However, human CD4 T cells retain expression of at least one activation marker, CD45RO, that is useful for identifying memory populations. In addition, memory CD8 T cells generally express elevated levels of the CD44 protein. Most memory cells are quiescent, but a small percentage of cycling memory cells can be found long after the initial response. It is not yet known what stimulates these cells or what role they play in the maintenance of memory. This is an extremely important issue, and scientists are investigating different possible mechanisms by which memory might be maintained. These include intermittent reactivation by very small amounts of antigen left in the body or encountered in the environment; crossreactive responses to other antigens; and nonspecific activation by cytokines produced during responses to other antigens. Further work is needed to identify the roles of different antigen-presenting cells and the need for CD4 memory cells to help maintain or reactivate B and CD8 memory cells.

Upon encounter with the initiating antigen, memory T cells are activated more efficiently than are naive cells, and they produce much greater quantities of effector molecules to eliminate the antigen. The molecular basis for activation differences between naive and memory cells is not yet known. Memory cells might also migrate differently throughout the body and interact differently with antigen-presenting cells, and further work is needed to define these areas. A better understanding of the factors required for potent, long-term T cell memory is critical not only for more effective vaccine development, but also for applications of immune tolerance in allergy, autoimmune disease, and transplant rejection.

B Cell Memory

Memory B cells develop in the germinal centers (GC) of the spleen and lymph nodes, where they undergo clonal expansion, somatic hypermutation, and antigenic selection. Our knowledge of the exact molecular details of these processes is limited. The current notion, based on cell labeling studies, suggests that B cells called centroblasts proliferate, mutate their antibody (Ig) genes, and give rise to centrocytes as they come out of cell cycle. Mathematical models of Ig mutation as a means of affinity maturation indicate that mutation and antigenic selection should proceed concurrently or, at least, that B cells should undergo several short rounds of mutation followed by antigen selection, suggesting shuttling between the mutating and selecting populations. None of the signals that control these differentiation steps are known. For instance, we have no idea which signals initiate and drive the proliferation of GC B cells, or which signals initiate hypermutation and cause transit from centroblast to centrocyte or *vice versa*. We do understand that the final stage of long-term rescue from apoptosis and entry into the recirculating memory pool is mediated by the CD40 ligand molecule, which is induced on T cells that surround the GC and interact with the antigen-presenting CD40-positive B cells.

One question that has proven intractable thus far is the identity of the molecular elements of the mutation machinery. This problem is currently under study in a number of labs. Identification of the mechanism may have a number of practical uses. For example, specific mutations might be targeted to create enhanced affinities or needed specificities. At a more general and practical level, the recent work of Rolf Zinkernagel raises the question of whether affinity maturation is necessarily a useful process in responses to complex and infectious organisms. First, protection against viruses requires that neutralizing antibodies be produced rapidly; we cannot wait for mutation to occur in a primary response. Interestingly, the primary antibodies produced in response to vesicular stomatitis virus were found to be of relatively high affinities in their germline states without mutation. Second, *in vivo* protection correlates more closely with serum antibody concentration than with avidity or on-rate. These provocative results indicate that future studies should focus more closely on the benefits of affinity maturation versus antibody concentration for immune protection.

Another central issue that is still unresolved is how B cell memory is maintained. Protection from infection is mediated by cells that can respond immediately with an effector function. For many infections, antibody is the most important protective factor, and it should preexist in the serum for optimal effect. We know that antibody production can continue for long periods after infection/vaccination and is derived from the B cells called plasma cells, which are most prevalent in the bone marrow. Although these cells are longer-lived than plasma cells found elsewhere, it seems likely that they require replenishment from precursors if they are to continue antibody production for up to 25 years. The process of differentiation of precursors into plasma cells is antigen-dependent, yet it is unclear that antigen always persists in the host for such long periods of time. Thus, the lifespan of bone marrow plasma cells deserves more investigation, as does their generation from precursor B cells.

The rules that govern the maintenance of memory B cells and lymphocyte homeostasis in general are still obscure. However, clues are emerging. For instance, the concept of follicular niches, which are thought to be different microenvironments that allow or impose different functional outcomes for B cells, is gaining more support and will be an important focus for future research. Follicular niches may be characterized by particular chemokine receptors, among other factors, and may provide a means for naive or memory B cells to gain appropriate access to antigen, perhaps as presented by the follicular dendritic cells that are thought to be crucial for B cell homeostasis. The characterization of gene knockout mice that show defects in lymphoid architecture, and in particular in GC development, will lead to an increased number of research tools available to understand the generation and maintenance of B cell memory. Such mice are already proving useful in addressing the role of follicular dendritic cells in immune memory.

Therapeutic Immunomodulation

In many clinical situations, the immune system fails to provide a response of sufficient strength or quality to fight infections or control tumor growth, suggesting that development of therapeutic procedures to upregulate the immune response would be useful. Depending on the cause of the insufficient immune response, different approaches will need to be developed. The failure of the immune system may depend directly on the specific clinical situation, such as the immunodeficiencies caused by HIV or measles virus, or immunodeficiencies associated with the growth of tumors, which, among other mechanisms, secrete suppressive factors such as TGFβ and IL-10. Immunodepression may also be associated with stress and injury, as in severe burns or following major surgery, or a result of the complex modification of the immune system associated with the aging process. These situations predispose the individual to opportunistic infections that are not observed in those with a normally functioning immune system.

Work of the past several years has clearly established that an insufficient immune response is not always secondary to a generalized or antigen-specific immunodepression or tolerance, but is often due to the type of immune response elicited by the infection, which may not be the optimal type to mediate resistance. This concept of immune deviation rather than immune depression is best represented by, but not limited to, the dichotomy between Th1- and Th2-type responses. Thus, approaches to upregulate immune responses can be either quantitative, focused on general or specific boosting of a depressed immune system, or can be qualitative, resulting in immunodeviation, such as switching the type of Th response or the antibody isotype. The use of adjuvants in vaccination should be emphasized among procedures to upregulate the antigen-specific response, including a focus on therapeutic vaccines aimed at treating infection postexposure or at treating established tumors. In the case of immune deviation, one particular challenge is posed by the observation that established Th responses are difficult to change, and it is important to develop methods that enable Th class switch.

Presently, many possible approaches to upregulate immunity exist, but their application will require an improved understanding of immunobiological regulation in humans and the establishment of appropriate modalities of treatment in both the preclinical and clinical settings. In particular, it should be expected that several of the following products or procedures will move into clinical practice: (1) cytokines, such as IL-2 to induce T cell expansion, GM-CSF to promote activation of antigen-presenting cells (APC), IL-4 to favor Th2 responses, and IL-12 and IGIF to favor Th1 responses; (2) co-stimulatory factors, such as the B7 family of CD28 ligands to promote T cell stimulation and expansion, and the CD40 ligand to activate B cells and APC; (3) adoptive transfer of APC, such as dendritic cells, that are purified, activated, expanded, and charged with specific antigens *in vitro* or are genetically engineered *in vitro* to express specific cytokines or costimulatory factors; and (4) adoptive transfer of antigen-specific T cells that are expanded *in vitro* or genetically altered to enhance

cytokine or costimulatory expression and improve *in vivo* functions. Conversely, approaches to downregulate immunity in allergy, autoimmune disease, and transplant rejection may involve (1) antagonism of costimulatory molecules, (2) antagonism of innate response mediators, or (3) deviation of an immune response by transfer of regulatory T cells or their products (Figure 4-3).

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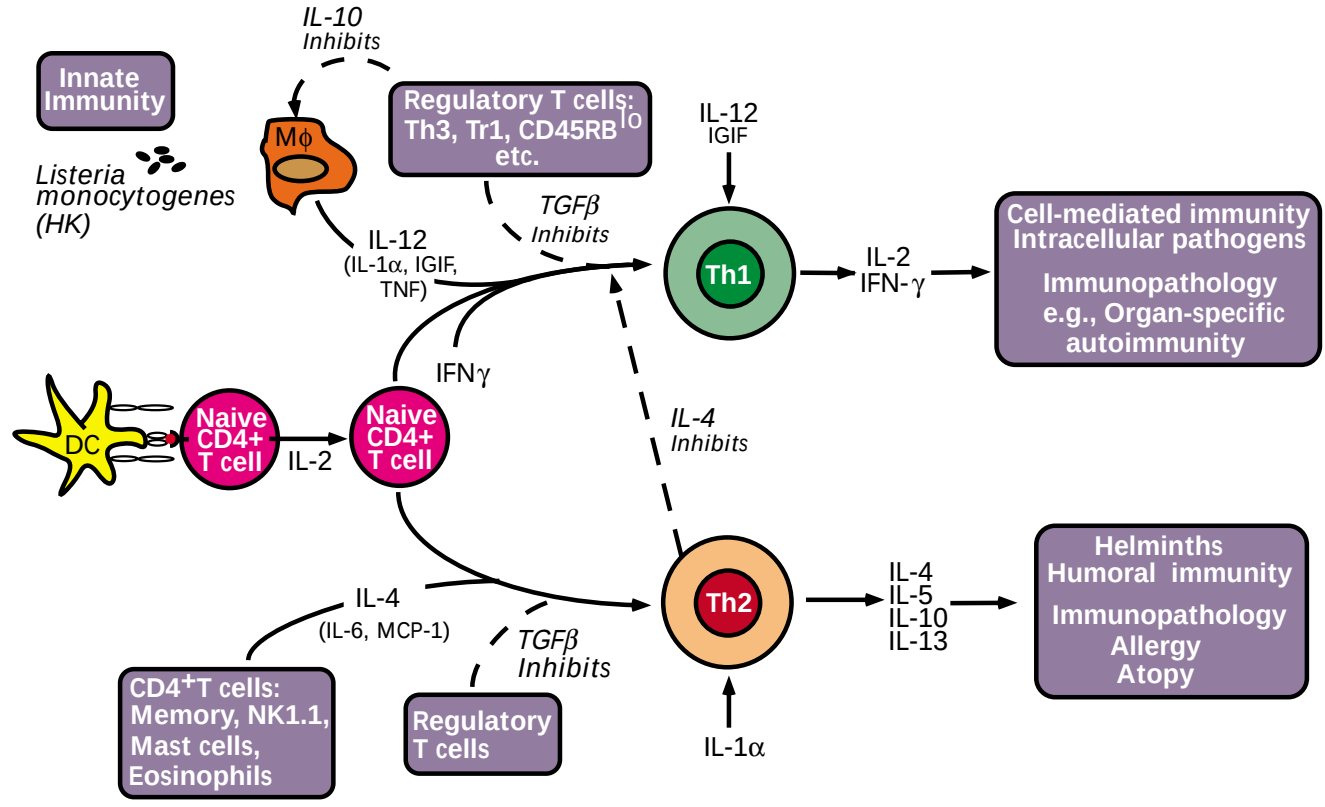

Figure 4-3. Regulatory T Cells Control Th1 and Th2 Responses

Source: O'Garra, A. Cytokines induce the development of functionally heterogeneous T helper cell subsets. *Immunity* 8:275–283, 1998; used with permission; copyright held by Cell Press.

Research Opportunities

T Cell Subsets

- Characterize site-specific development and regulation of CD4 and CD8 T cell subsets, particularly in the lung, intestinal tract, and brain
- Identify surface marker proteins that distinguish Th1- and Th2-type cells to facilitate *in vivo* monitoring of subset dominance during immune responses; define the molecular basis for the development of Th1 and Th2 phenotypes
- Define the cellular and molecular bases for downregulating established Th1- or Th2-type immune responses
- Characterize regulatory T cell subsets and the molecular basis for their activities
- Translate basic understanding of Th cell subsets for application to specific diseases, including infectious disease, autoimmunity, allergy, and cancer

B Cell Differentiation

- Identify the mechanisms of somatic mutation of immunoglobulin genes
- Determine the relative importance of antibody concentrations and affinity in human defenses against microbes
- Identify the relative roles of the IgE and IgG isotypes in allergy and anaphylaxis, and IgA in mucosal protection, in rodent and primate disease models
- Develop better experimental models for identifying the factors that regulate human isotype switching *in vivo*
- Characterize the enzymes and DNA binding proteins involved in Ig switch recombination and the role played by germline C_H transcripts in isotype switching

Immune Memory

- Determine the molecular events responsible for the generation and maintenance of T and B cell memory
- Define the roles of particular microenvironments on memory T and B cell migration, differentiation, and lifespan

Interactions of the Innate and Adaptive Immune Systems

- Increase understanding of the role of innate immune system components and the requirements for cytotoxic CD8 T cell activation
- Define pathogen-derived molecules that interact with the cells of the innate immune system and stimulate production of immunologically relevant molecules involved in initiating and directing the immune response

Enhancement of Immunity

- Develop therapeutic vaccines that upregulate or modify established human immune responses or overcome peripheral tolerance to treat persistent infections or tumors
- Develop procedures to permit better *in vivo* survival of genetically engineered T cells and dendritic cells to prevent their rejection upon recognition of the transgene or vector gene products
- Characterize the plasticity of immune responses; define the redundancy of pathways involved in accessing and regulating the immune response to identify alternative approaches for activation and inhibition

O'Garra, A. Cytokines induce the development of functionally heterogeneous T helper cell subsets. *Immunity* 8:275–283, 1998.

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Inflammation and Host Defense

5

Overview

As has been recognized for centuries, acute inflammation is characterized by *rubor* (redness or erythema), *dolor* (pain), and *calor* (heat), such as are found with an infected tooth or wound. Inflammation is essential for early host defense, but an unregulated response can be quite harmful to the host. The effector mechanisms of inflammation are focused on recognizing any perturbation of homeostasis in the whole organism, and then on organizing and executing an effective strategy for a return to homeostasis. This function requires the coordinated efforts of several different cell types, which originate from hematopoietic stem cells in the bone marrow and are characterized by their circulation throughout the body. These cells are also able to enter and migrate through solid tissues, either in attempts to "scout" for sites of trauma, burn, infection, foreign body, or other tissue injury or in response to specific signals from the injured or infected tissue. This process of destruction of invaders and repair of host tissue is called inflammation. The primary cells involved in these acute responses are the white blood cells, or leukocytes, which include monocytes, neutrophils, basophils, eosinophils, and lymphocytes. Tissue cells such as macrophages and mast cells also participate. The responses of these cells to an inflammatory stimulus can occur in a nonimmune host, but they are accelerated and amplified by a variety of soluble factors, such as antibody, complement, cytokines, and chemokines, which are produced by or markedly enhanced by specific adaptive responses of T and B cells. Thus, inflammation is a common pathway for host protection that integrates signals from both the innate and the adaptive immune systems.

Although host defense against acute insult is the evolutionary purpose of this arm of immunity, the inflammatory response can have a markedly deleterious effect on the host when not appropriately regulated. If the stimulus cannot be eliminated—because it is an infectious agent such as the tuberculosis bacterium, which protects itself by taking up residence inside host cells; because it is a foreign body that cannot be eliminated; or because it is unknown, as in rheumatoid arthritis—the signals that initiate the inflammatory response continue to be generated. This chronic inflammatory response differs in some respects from the acute and rapidly resolved response because activated macrophages and lymphocytes play a more prominent role than granulocytes in the chronic inflammatory response. The pathologic hallmark of chronic inflammation is the granuloma, a collection of macrophages and lymphoid cells that serves to isolate the inflammatory focus from the rest of the body. Often, fibroblasts become involved in chronic inflammation as well, leading to fibrosis, an increased and disordered deposition of extracellular matrix, which further serves to wall off the inflammatory focus.

The inflammatory response has been implicated in the pathogenesis of diseases as diverse as diabetes, atherosclerosis, Alzheimer's disease, reperfusion injury, and even cancer, as well as in the pathology that follows infectious meningitis, in postinfection syndromes such as rheumatic fever, and in idiopathic autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus. The centrality of the inflammatory response in these varied disease processes makes its regulation a major goal for the prevention, control, or cure of human disease. To this end, a detailed understanding of the normal and pathological mechanisms that generate and regulate the inflammatory response is required, as many of these processes are still poorly understood. Drugs such as aspirin and the so-called nonsteroidal anti-inflammatory drugs are a common part of everyone's medicine cabinet. Recently, basic research into the mechanisms of inflammation has identified new targets for anti-inflammatory drugs. Drugs have been developed that inhibit leukocyte migration to sites of inflammation or block key enzymes in the inflammatory response, such as cyclo-oxygenase, the stress-activated protein p38, and leukocyte tyrosine kinases. Although not yet commonly available, several of these drugs are undergoing clinical trials.

Major strides in the past 5 years provide a strong foundation for a detailed model of leukocyte accumulation at sites of inflammation. This model is supported by a wealth of experimental detail obtained both *in vitro* and *in vivo*. Soluble chemoattractant and leukocyte-activating molecules have been identified and characterized, and receptors for both new and previously characterized soluble mediators have been cloned. Significant advances have occurred in understanding the molecular events that lead to leukocyte activation by bacterial peptides, lipopolysaccharide, and complement components. The molecular processes of cell adhesion, locomotion, and generation of substances toxic to microorganisms or host tissues are understood in greater detail, providing a much clearer picture of how an inflammatory response occurs. This chapter summarizes some key advances and highlights the most pressing issues for the next 5 years.

Innate Immunity

Innate immunity comprises a broad area of host defense, including all systemic host protective mechanisms that do not require prior exposure to antigens and are thus not antigen-specific. Innate immunity is the primordial system for self-nonself discrimination and for the detection of danger to the host. The complement cascade and circulating proteins such as C-reactive protein, mannose-binding protein, and amyloid P component are soluble effectors of innate immunity. The "professional" phagocytes that include polymorphonuclear (PMN) leukocytes (neutrophils, basophils, and eosinophils), monocytes, and macrophages are the cellular elements of innate immunity, as are mast cells and the specialized lymphocytes known as natural killer (NK) cells.

Important interactions occur between the soluble and cellular components of the innate immune system. The soluble factors bind to invading pathogens, and the phagocytes then recognize such

complexes and destroy them. Phagocytes also have membrane-bound mannose receptors, scavenger receptors, and lipopolysaccharide receptors, which can directly recognize and ingest some invading pathogens through bacteria-specific carbohydrate or lipid molecules. Innate immunity is a venerable area of research, and information about the structure and function of many of the soluble factors and cellular receptors for complement fragments and mannose has been available for a number of years. More recent progress in the application of modern cellular, genetic, and molecular techniques has provided a much more comprehensive understanding in this area. Key advances include the discovery of receptors for self-nonself discrimination by NK cells, identification of an extensive scavenger receptor family on phagocytes, increased understanding of how intracellular pathogens subvert components of innate immunity to infect the host, and the role of complement in enhancing B lymphocyte responses.

The innate immune system also interacts in an intricate manner with the components of adaptive immunity. The innate immune system can be seen as a set of highly integrated layers of response to any threat. Evolutionarily primitive systems, such as lysis *via* the complement cascade, are rapid but relatively easy for potential pathogens to evade. The most advanced precise mechanism of host defense is the adaptive immunity provided by antigen-specific mechanisms of T and B cells. None of the host defense mechanisms protect the host independent of the other systems. For example, phagocytic cells are highly dependent on complement for activating and discriminating signals. Adaptive immunity is dependent on complement and phagocytes for antigen recognition and effector functions. Moreover, antibodies produced by the adaptive immune system augment the functions of the underlying innate mechanisms, such as antibody-dependent complement-mediated lysis or antibody-dependent phagocytosis. A simple laceration with exposure of normally nonpathogenic skin bacteria to immune mechanisms will lead to cooperative responses among platelets, neutrophils, mast cells, antibodies, and complement.

Cellular and Soluble Mediators of Inflammation

The normal inflammatory response occurs only at sites of homeostatic perturbation caused by a potential pathogen, injection of a foreign body, or wounding. The response is spatially and temporally constrained, and its purpose is not only to restore homeostasis but also to prevent systemic spread of both the potential pathogen and the potentially host-damaging mediators of inflammation. First, there must be a mechanism to recognize perturbation of homeostasis and to communicate this understanding to the inflammatory effector cells. This is achieved through a wide variety of molecules, including components of the clotting cascade, bacterial peptides, carbohydrates and lipids, complement fragments, and a large number of soluble molecules produced by leukocytes and epithelial cells at the site of inflammation (Table 5-1). These soluble molecules are synthesized and/or secreted in response to the inflammatory stimulus. They function both to initiate the accumulation of leukocytes and to amplify the inflammatory response.

Table 5-1. Chemoattractants

CLASSICAL CHEMOATTRACTANTS	
Leukotriene B_4	
Platelet-activating factor	
N-formyl peptides	
C5a	
CHEMOKINES	
α (CXC)	β (CC)
IL-8	MIP-1α
GROα	MIP-1β
GROβ	RANTES
GROγ	MCP-1
NAP-2	MCP-2
ENA-78	MCP-3
GCP-2	I-309
γIP-10	
PF-4	
NAP-4	
Mig	

Mediators of inflammation can also influence the process of angiogenesis, which creates new blood vessels from existing ones. Angiogenesis occurs in response to ischemia or to increased metabolic demand created by increased cell turnover and proliferation. Thus, significant angiogenesis most frequently accompanies chronic inflammation, when the continued influx and turnover of cells lead to new vessel formation. A large number of angiogenesis factors have been isolated within the past few years, and some endothelial receptors involved in stimulation of angiogenesis have been identified. Although clinical trials have been initiated using antiangiogenic factors to limit tumor growth, little has been done with these agents in inflammation, because angiogenesis in this case is thought to be a late consequence rather than an initiating factor in the disease.

A strong molecular understanding of certain parts of the inflammatory response, such as the complement and coagulation cascades, existed as early as 10 years ago. The past few years have seen the concerted application of molecular biology to this field, and multiple new chemokines, cytokines, and their receptors have been cloned, as have several new enzymes involved in arachidonate metabolism. Signaling cascades used by the chemokine and cytokine receptors to activate inflammatory cells have been partially unraveled. New understanding of how bacterial products such as fmet-leu-phe and lipopolysaccharide mediate their inflammatory effects has also been achieved. Together, these advances have provided new insights into both the overlapping and the specific functions of these important mediators of inflammation. Key advances include identification of the CC and CXC chemokine families, discovery of CCR5 and SDFR as coreceptors for HIV infection, demonstration of extensive sharing of receptors by different chemokines, identification of functional interactions among multiple mediators, appreciation for extensive use of GTPase-associated receptors in leukocyte signaling, and early development of specific inhibitors of inflammation. Many opportunities exist for research in the immedi-

ate future, with important implications for the development of new drugs that regulate inflammation.

Cell Adhesion and Migration

Because the cellular effectors of the inflammatory response normally circulate in blood, they must cross the endothelial barrier and then migrate through extracellular matrix to enter an extravascular site of inflammation. This exit from the bloodstream and migration into tissue must occur in vessels close to the inflammatory site, which implies that specific recognition mechanisms exist for the interaction of leukocytes with endothelium overlying an infection, foreign body, or wound. Work in the 1980s revealed the existence of tissue-selective mechanisms of leukocyte-endothelial recognition, suggesting an explanation for leukocyte recruitment in inflammation. Advances in techniques for culturing endothelial cells and for examining interactions between leukocytes and endothelium led to the identification of molecules involved in these tissue-specific interactions between leukocytes and endothelium *in vitro*.

Exciting *in vivo* and *in vitro* approaches in the past few years have led to a powerful and detailed model for leukocyte-endothelial interaction based on a large amount of detailed experimental data (Figure 5-1). This multistep process

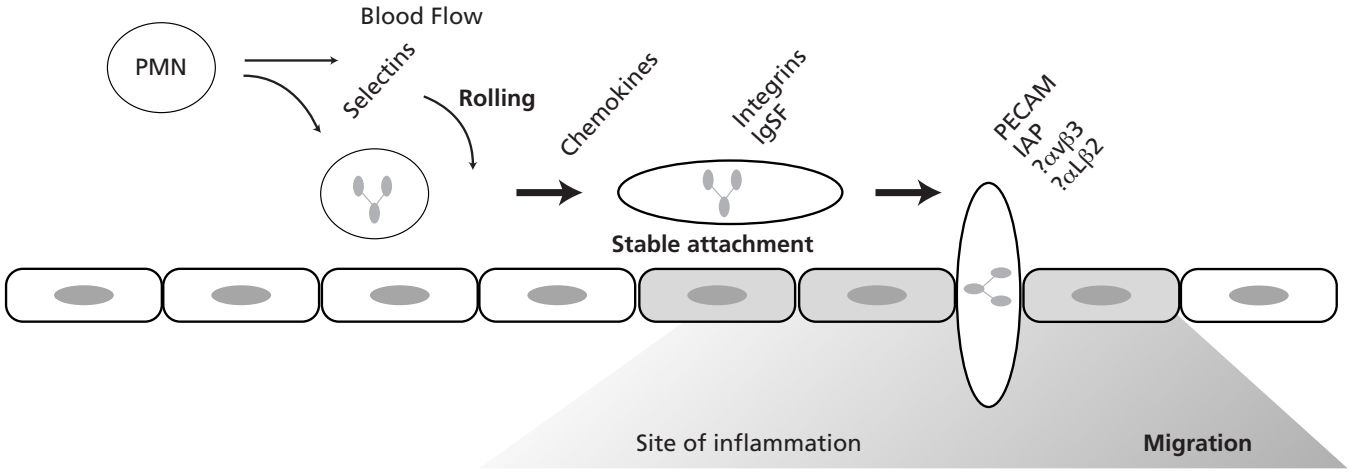

Figure 5-1. Polymorphonuclear neutrophil (PMN) interactions with the endothelium. There are three basic steps in the process by which PMNs move from the blood into the extracellular space. First, selectin molecules on both the endothelium and the PMN recognize carbohydrate ligands on the other cell type. These selectin-carbohydrate interactions have rapid on and off rates, which, under conditions of blood flow, lead to PMNs rolling along the endothelial cells. The rolling leukocytes are exposed to chemokines, which interact with GTPase-coupled receptors. This activation step leads to enhanced integrin avidity and stable, nonrolling interaction of PMNs with endothelial cells through integrin recognition of immunoglobulin superfamily (IgSF) members expressed on the endothelial surface. The PMNs then move through the interendothelial junctions, a process mediated by the IgSF members, PECAM and IAP, but potentially requiring integrins as well. PMN contacts with the endothelium during penetration must be very close, because electrical resistance across the endothelial monolayer is not lost during PMN transmigration. Reproduced from Brown, E.J. *Trends in Cell Biology*. Elsevier Science Ltd. 7:289–295, 1997; copyright 1997, with permission from Elsevier Science.

involves an initial interaction between leukocytes and endothelium that is rapid but transient and leads to leukocyte rolling on endothelium overlying sites of inflammation. The rolling interaction allows the leukocyte to sensitively "search" for inflammatory mediators, especially IL-8 and other chemokines, which in turn activate additional adhesive interactions mediated by integrins and their ligands. Finally, the leukocytes move to interendothelial junctions, where they emigrate through the endothelium without damaging the integrity of the endothelial barrier. Thus, the essential role of cell adhesion in leukocyte activation has been elucidated, and genetic, cellular, and biochemical approaches have produced a wealth of complementary data. Key recent advances include the development of assays for adhesion under conditions that mimic blood flow, *in situ* videomicroscopic dissection of leukocyte-endothelial interactions, the role of chemokines in activating integrin-mediated adhesion, identification of selectin ligands, and the discovery of cooperation among selectins and integrins in cell adhesion processes. Future research will extend these studies to an understanding of the intracellular events that regulate adhesion and migration and to development of drugs that affect leukocyte accumulation at sites of inflammation and infection.

Signal Transduction in Inflammation

In the absence of inflammation, leukocytes circulate in the bloodstream in a quiescent state. They are nonadhesive and not very phagocytic; they consume little oxygen and make little RNA or protein. However, at sites of inflammation these cells are dramatically changed. They become highly adhesive and phagocytic, and oxygen consumption is markedly increased. Some oxygen is utilized by an NADPH oxidase to create toxic, high-energy oxygen metabolites critical for killing bacteria. Messenger RNA and protein production is markedly enhanced, leading to the production of soluble mediators of inflammation, including tissue-damaging proteases. This marked phenotypic change is called leukocyte activation. Teleologically, leukocyte activation must be confined to sites of inflammation to prevent systemic damage by the potent, nonspecifically toxic proteases and oxygen radicals generated by activated leukocytes.

As mentioned, activation occurs in response to a wide variety of substances, including bacterial peptides, extracellular matrix components, complement components, lipid metabolites, and immune complexes found predominantly only at inflammatory sites. A coordinated series of biochemical steps transduces cell surface receptor signals into the various effector functions required of the activated leukocyte. Previous understanding of the molecular mechanisms of leukocyte activation was largely limited to understanding the assembly of the multicomponent NADPH oxidase. The past 5 years have seen rapid advances in understanding signaling cascades in inflammatory cells, thus providing numerous targets for future pharmacological modification of the inflammatory process. Key advances include the identification of tyrosine kinase cascades in Fc receptor-mediated activation by immune complexes, discovery of the roles played by MAP and GTPase-activated kinases in leukocyte activation, elucidation of the importance

of leukocyte adhesion, and a greater understanding of receptor cooperation in leukocyte activation.

The Resolution of Inflammation

Restoration of homeostasis after an inflammatory insult leads not only to cessation of leukocyte influx into the site but also to rapid removal of the leukocytes present. This may be followed by the accumulation of nonleukocytic cells and deposition of increased amounts of altered extracellular matrix, leading to scarring. This late phase of the inflammatory response is still poorly understood at a molecular level. The mechanisms by which the leukocyte number is downregulated at inflamed sites, by which the normal population of resident cells is restored, and by which the extracellular matrix is remodeled are only partially understood. Apoptosis of the inflammatory cells is an important component of the resolution of inflammation, and it probably occurs through a Fas-mediated mechanism. Apoptotic cells are then recognized, phagocytosed, and eliminated by resident tissue macrophages. This process can occur through several different receptor systems, including scavenger receptors, integrins, and phosphatidylserine receptors. In contrast, emigration from the inflamed site may be an important means for the removal of macrophages and lymphocytes during resolution of inflammation.

A key role for leukocyte signal transduction in the resolution of inflammation is illustrated by the so-called *motheaten* mutation in one mouse strain. The salient feature of these mice is that they fail to resolve inflammatory responses. It was recently demonstrated that these mice have a genetic defect in expression of a leukocyte-specific tyrosine phosphatase, called SHP-1, which plays a critical role in leukocyte signal transduction. Other key advances include the development of sensitive techniques to detect the phagocytosis of apoptotic cells *in situ* and the demonstration that macrophage phagocytosis of apoptotic cells is used in *C. elegans* and drosophila as well as in higher eukaryotes, thus facilitating research on the specific pathways used.

Many of the effector mechanisms associated with inflammation are nonspecific, such as the generation of reactive oxygen and nitrogen metabolites and secretion of hydrolytic enzymes. Therefore, these responses are damaging to host tissue as well as the agent inciting the inflammation. In the short term, this is beneficial because it can destroy the invader and, by damaging surrounding host tissue, amplify the acute inflammatory response. However, if inflammation is not promptly and efficiently resolved, continued destruction of host tissue occurs, leading to irreparable tissue damage, scarring, and loss of function. Rheumatoid arthritis, with the destruction of multiple joints due to unresolved inflammation, is a classic example of the damage that can occur. Inflammatory bowel disease, diabetes, glomerulonephritis, and multiple sclerosis are other human diseases in which failure to resolve inflammation properly leads to significant organ damage. Clearly, much remains to be learned about the critical stages of the resolution of inflammation and potential approaches for human therapy.

Research Opportunities

- Develop animal models of human inflammatory diseases and conduct *in vivo* analyses of inflammatory mediators and receptors
- Develop therapeutic drugs based on newly discovered mediators of inflammation
- Further define the molecular mechanisms of leukocyte adhesion and migration to understand transit through the endothelium from bone marrow to the blood, and from the blood into affected tissues; clarify the role of proteolysis in migration through the extracellular matrix
- Define molecular mechanisms for the regulation of leukocyte accumulation at inflammatory sites and for reversal of leukocyte activation and inflammation
- Characterize the signal transduction pathways important in leukocyte activation
- Develop methods to augment innate immunity in the treatment of infectious diseases and determine the specific components of innate immunity that optimize adaptive immune responses

Many experimental models have been developed for the study of inflammation, ranging from the instillation of sterile inflammatory agents such as casein, thioglycollate, or lipopolysaccharide into experimental animals, to infection with many different free-living bacteria. The advent of gene knockout technology has made possible dissection of the complex pathways involved in the generation of inflammation *in vivo*. In addition, some mutant mice, such as the motheaten discussed above and the TGFβ gene knockout, develop spontaneous inflammation, providing some insights into the normal regulation of the inflammatory response. Hypotheses concerning the molecular mechanisms involved in initiation and regulation of inflammation will be testable *in vivo* using such model systems. Considerable future work will be required to dissect the molecular pathways that control the inflammatory response.

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Immediate Hypersensitivity and Allergy

6

Overview

The allergic response is a central mechanism in some of the most common disorders affecting humans, such as asthma, hay fever, atopic dermatitis, and food allergy. It may also result in systemic anaphylaxis, the most severe form of allergic reaction, which can cause cardiovascular collapse and death. The allergic response participates in diseases as diverse as serum sickness, drug or insect sting reactions, reactions to X-ray contrast dyes, and autoimmune diseases. Nearly one-third of the U.S. population now shows evidence of sensitization to environmental allergens.

The allergic (atopic) response is a form of adaptive immunity in which cells of the *afferent/initiator limb* of the response recognize a foreign substance and produce allergen-specific immunoglobulin E (IgE) antibodies. The cells central to the afferent limb include antigen-presenting cells (APC), Thl and Th2 cells, B cells, mast cells, and possibly basophils as a source of priming cytokines. Upon subsequent exposure and IgE binding to the same foreign antigen, IgE:antigen complexes bind to high-affinity IgE receptors on mast cells and basophils, activating the cells to release soluble mediators. This forms the *efferent/effector limb* that induces a very rapid biological readout, which is a specialized form of inflammation. Importantly, the efferent limb can also be amplified and even elicited by non-antigen-specific stimuli such as cytokines or complement components, providing a role of the same pathway in the innate host responses that precede (in newborns) and parallel adaptive immunity. Cells central to the efferent limb of the allergic response include Th2 cells, mast cells, basophils, and eosinophils.

Features that are of particular relevance to development of the *afferent limb* include:

- sensitization to environmental allergens at the skin or mucosal surfaces in the context of environmental adjuvant-like activities;
- specialized skin or mucosal APC that initiate the immune response in a genetically constrained manner;
- local inflammatory cells that modulate APC expression of key costimulatory molecules and thereby shape the interactions between APC and antigen-specific T and B cells;
- activation of T cells to provide help for IgE production and to participate in the efferent limb by production of specific allergy-promoting cytokines and chemokines; and
- B cell proliferation and differentiation into a dominantly IgE-committed pathway to generate memory B cells that can produce high levels of IgE upon restimulation.

The immediate allergic inflammatory response, or *efferent limb*, is invoked upon allergen reexposure. The efferent paradigm includes the following important concepts:

- inflammation can be a beneficial/protective host response, mediated by both the innate and adaptive immune systems, after environmental insult; it becomes detrimental only when it is inappropriate in intensity or specificity;
- inflammatory responses are highly complex, involving cytokine-mediated hematopoiesis to increase circulating numbers of the relevant effector cell populations, cellular adhesions that retain pertinent cell types at the microvasculature, cellular migrations that move effector cells to the extravascular sites of injury, and soluble mediators that are released to help confine or aggravate the insult, during physiologically beneficial or pathobiological responses, respectively;
- effector cells are regulated through receptor-activated signal transduction pathways to release preformed or newly synthesized mediators at the inflammatory site; and
- phenotypic alterations occur in each cell type at an inflammatory site, in the absence of cellular transformation; this is the hallmark of an inflammatory response. The therapeutic challenge is to be able to intervene in the basic pathobiology without preventing needed physiological repair and without inducing cellular transformations.

Immunotherapy for immediate hypersensitivity disorders is still based on empiric observations from the early 1900s that suggested that repeated subcutaneous injections of allergens improved symptoms. The technique relies on gradually increasing doses of the natural allergens. Other than identification and standardization of some of the allergens, this approach has changed little. While it has clear clinical benefit in upper airway and insect sting allergy, its role in lower airway allergy (asthma) remains controversial, and it is not efficacious in food allergy or atopic dermatitis. Furthermore, side effects are common and troublesome. Thus, there is a great need for new approaches that may be broadly effective in immediate hypersensitivity disorders as well as approaches that are directed toward specific allergens. New therapies can be predicted to result from our vastly improving knowledge of the cellular and molecular aspects of the immediate hypersensitivity response.

Molecular cloning studies have resulted in considerable progress in defining the primary structures of allergens and their biological functions. Epitopes that activate B and T cells and specific allergen determinants that elicit dominant IgE responses are being identified; further studies are needed in these important areas of research. Development of high-level expression systems for recombinant allergens will facilitate basic research and yield new recombinant products for diagnostic and therapeutic purposes. Evaluation of allergen-based therapies will require large clinical trials for efficacy testing and companion studies that identify mechanisms of action. Detailed studies on the epitope specificity, cytokine production, and ontogeny of responses in different populations of allergic individu-

als are required. Many new approaches to immunotherapy should be tested, such as peptide vaccines that prevent allergic T cell responses, and development of vaccines with adjuvants or other biological response modifiers that minimize Th2 development.

Environmental Aspects of Allergic Inflammation

The natural history of allergy and asthma is not well understood. Inhaled allergens are the most common causes of allergic IgE responses in atopic humans. Both respiratory infections and environmental factors such as tobacco smoke, dust mites, animal dander, insects, pollens, and spores may facilitate the induction of allergic sensitization. Exposure to allergens in conjunction with infections or air pollutants in infancy may determine the degree of sensitization in later life. Food allergens are apparently more important than inhalant allergens in systemic anaphylactic reactions and in allergic skin disorders in young children. Sensitization to both food and inhalant allergens is a major risk factor for asthma, seasonal and perennial rhinitis, and atopic dermatitis. Epidemiologic data suggest that indoor allergen exposure has played an important role in increasing the prevalence of asthma in the past 20 years, although other environmental changes have occurred in parallel with changes in housing. Thus, it is essential to design future studies to compare exposure to indoor allergens with other aspects of behavior, diet, and environment that could alter the immune response to allergens. Improved methods for quantitating allergen and other relevant exposures are needed to better define the risk of such exposures.

Genetics of Allergy and Immediate Hypersensitivity

Substantial progress has been made in evaluating the genetic controls that regulate atopic disease. However, unlike diseases linked to single genes, such as cystic fibrosis or sickle cell anemia, the magnitude of the risk attributable to a single gene in allergy is low. Furthermore, although the proportion of affected people is high, disease is quite variable across different populations, and the replication of experimental or therapeutic results is often problematic. Therefore, rigorous epidemiologic studies are needed to sort out the complexities of variable gene:environmental interactions, as well as the assessment of multiple disease parameters in defined experimental contexts.

The extraordinary progress of the Human Genome Project has facilitated genome-wide searches for putative genes associated with atopy (asthma, allergic rhinitis, atopic dermatitis). Current approaches include the analysis of "microsatellite" DNA polymorphisms, sequencing of allelic variants associated with the expression of allergic disease, and fine mapping of chromosomal regions to which atopy genes have been localized. Both conventional linkage studies, such as sib-pair analyses, and association studies between candidate genes, the allergic response, and the allergic type or specificity are needed. A deeper understanding of the molecular mechanisms of cellular activation and gene transcription is also needed to facilitate assignment of genetic abnormalities with function of the gene products. For example, one goal is to define and compare genotypes/phenotypes

involved in the regulation of Thl and Th2 cytokine production.

Cellular Components of Allergic Responses

Antigen-Presenting Cells

Allergy is generally initiated by antigen presentation to CD4 T cells of the Th2 type, which then provide help for B cell IgE production. In genetically predisposed individuals, the availability and functional capacity of particular APC are likely to play important roles in skewing T cell responses to the Th2 type and in the regulation of allergic responses. Mucosal dendritic cells (DC) and skin epidermal Langerhans cells are the principal resident APC at sites of allergen exposure. In addition, macrophages can play a critical role by secreting cytokines, chemokines, eicosanoids, prostanoids, and other soluble mediators that help shape the specific lymphocyte response. DC are derived from stem cells in the bone marrow, and they undergo a transition phase associated with efficient antigen sequestration and processing in peripheral tissues. Their APC function matures with subsequent migration to regional lymph nodes. Mature DC uniquely provide the accessory molecules required to elicit primary T cell responses. The effects of environmental factors such as pollutants, viral infection, and allergen exposure on mucosal APC trafficking and function need to be better understood. Control of allergic inflammation will also depend on increased knowledge of the particular APC types involved in generating allergic activation and the local cytokine milieu that contributes to Th2 versus Thl differentiation. Because the allergic phenotype is the net result of the quantity and quality of T cell responses after APC activation, understanding the control of expression of various adhesion and costimulatory accessory molecules on APC is an important future goal.

Effective antigen presentation and activation of CD4 T cells require processing of internalized allergen protein with subsequent presentation of its peptide fragments bound to MHC class II molecules. MHC class II is expressed constitutively on DC, B cells, and macrophages, but it can be induced on many other cell types by proinflammatory cytokines. High-affinity IgE receptors (FcεRI) are also expressed on APC surfaces, and they can capture and internalize specific allergen:IgE complexes to enhance allergen presentation. The physiological role of this process needs to be defined more fully. A detailed characterization of allergenic peptide epitopes and their sequence motifs critical for efficient binding to MHC class II molecules is also needed to facilitate novel therapeutic approaches to interrupt allergen presentation. The role of CD8 T cells that respond to allergen:MHC class I presentation is not yet clear. It is possible that such cells, perhaps those that express γδTCR, may downregulate CD4 T cell and DC function.

T Cells

The allergic inflammatory response is driven by the overproduction of Th2-type cytokines such as IL-4, IL-5, IL-10, and IL-13. Th2 cells enhance B cell growth and differentiation and support IgE production. They also promote mast cell, basophil, and eosinophil growth and differentiation and selectively recruit lymphocytes, basophils, and eosinophils

to sites of allergic responses. A strong Th2 response to allergen thus represents an inappropriate and detrimental adaptive response to nonpathogenic environmental antigens. In nonallergic individuals, the response to such antigens is predominantly protective, characterized by Th1-type cytokines, such as IL-2 and IFNγ, which suppress allergic inflammation. The factors that determine the Th1 *versus* Th2 dominance of the T cell response to allergens have not yet been identified and merit intense investigation.

B Cells

Allergen-activated B cells proliferate and undergo isotype switching from IgM to IgE upon appropriate stimulation by T cells. This switch involves deletion of a large segment of DNA between the immunoglobulin μ and ε heavy chain loci and results in the positioning of the antigen combining region (VDJ) next to the ε constant region for the synthesis of IgE RNA and protein (also see Chapter 4). The mechanisms that regulate μ to ε isotype switching involve nonhomologous DNA rearrangement and are not yet well understood. Both IL-4 and IL-13 promote the switch to IgE, and switching events specific to IgE may serve as targets for intervention in allergic responses. Interestingly, the single functional human ε gene produces a family of ε transcripts as a result of alternative RNA splicing, including two transcripts that encode membrane IgE forms and several that encode secreted IgE forms. The mechanisms that regulate alternate splicing are not well understood, and functional differences in the final IgE proteins have not yet been well defined. IgE-producing B cells are preferentially localized at mucosal surfaces, but it is not known whether mucosal localization results from IgE B cell-specific trafficking interactions, or whether local factors induce IgE B cells preferentially in mucosal tissues. Some evidence suggests that IgE-producing B cells may have a prolonged half-life, but the mechanisms for isotype-dependent lifespan have not been determined. A first generation of therapeutics designed to interrupt IgE binding to FcεRI has shown some efficacy. Further refinements of these methodologies as well as their use with other forms of intervention are likely to yield improved approaches.

Mast Cells

Mast cells clearly play an important role in both the initiation and perpetuation of IgE-dependent responses such as anaphylaxis and allergic asthma. Mast cells express IgE receptors and release potent soluble mediators upon allergen:IgE binding (Figure 6-1). Recent findings suggest that mast cells also function in innate immunity and as immunoregulatory cells, by producing a variety of cytokines with potentially diverse regulatory actions in inflammatory and immune reactions, and also by serving as APC. Increasing evidence indicates that these cells participate in a wide variety of clinically significant IgE-independent responses, including tissue remodeling, angiogenesis, wound healing, and the host response to cancer. The preformed mediators found in mast cell secretory granules, such as the proteolytic tryptase and the membrane-derived lipid mediators of the cysteinyl leukotriene family, appear to be direct contributors to the pathobiology of asthma. There is growing appreciation that mast cells are exceedingly plastic in both phenotype and function and that many different stimuli can promote or suppress

the mast cell phenotype and influence its pattern of proinflammatory/immunoregulatory mediator release.

Many significant gaps remain in our understanding of mast cell development, phenotypic variability, and function in human health and disease. It is known that the molecule called stem cell factor is critical for normal mast cell development, but other factors that influence vascular egress, tissue retention, tissue migration, proliferation, function, and lifespan are not well defined. IgE receptor-mediated release of soluble effector molecules has been well studied. However, IgE-independent mast cell functions, such as responses to bacterial infections and IgG- and complement-dependent inflammation, and their roles in immunologically nonspecific acute and chronic inflammation and tissue remodeling remain to be elucidated. Such studies may help define mast cell function in diseases such as scleroderma and rheumatoid arthritis.

One promising area for further research is the manipulation of mast cell mediator release as a therapeutic approach to control human disease. More information is needed on the intracellular signal transduction pathways responsible for mediator secretion, on mast cell surface receptors, such as FcγRIIB and gp49B1, which are known to inhibit mediator release, and on the particular stimuli that elicit or prevent the release or new synthesis of individual mediators. Efforts to establish functionally responsive human mast cell lines *in vitro* will aid in the study of phenotype and function. It is now possible to generate functional, nontransformed human mast cells from umbilical cord blood-derived progenitors for definitive studies *in vitro*, although large amounts of expensive and relatively rare cytokines are required for the production of substantial cell numbers. Ongoing work is focused

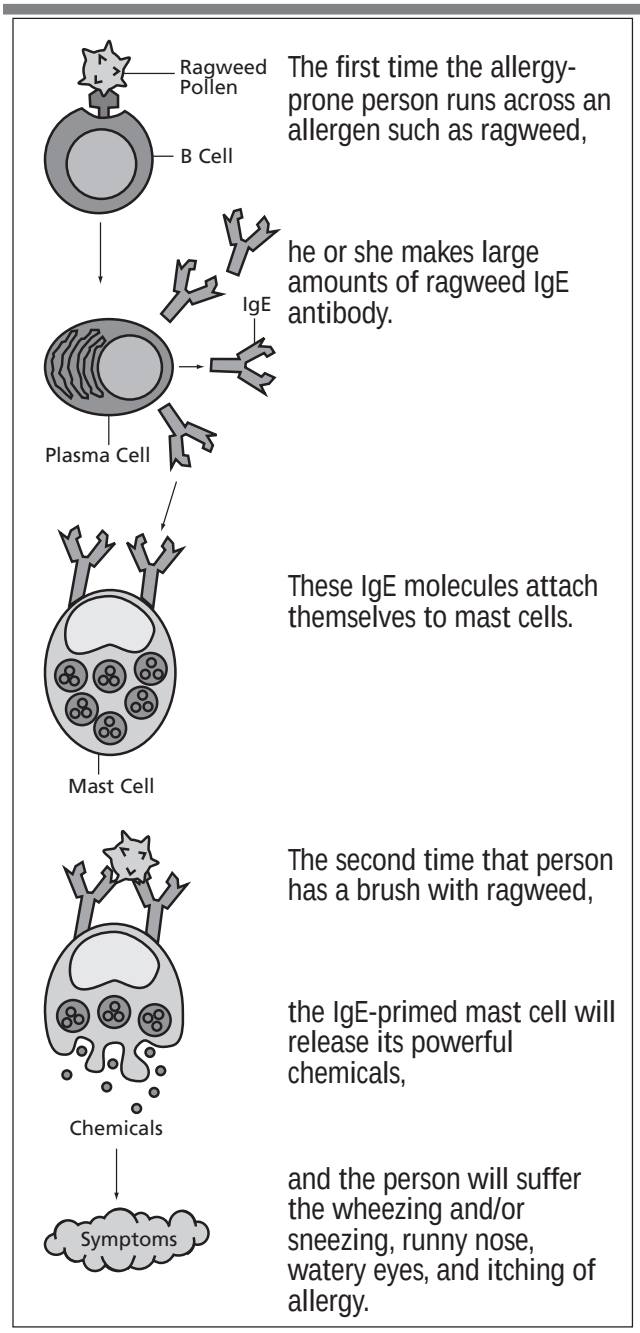

Figure 6-1. Mast Cell Degranulation

Source: From the NCI/NIAID pamphlet *The Immune System—How It Works*

on defining functional mast cell receptors and mechanisms that regulate secretory granule tryptase expression.

Basophils

Studies on human basophils have been hampered by inadequate facilities to generate purified cells from well-characterized donors. However, progress has occurred in several areas, in part derived from animal work. Adhesion molecules, such as VLA4/VCAM-1, were found to be involved in the selective recruitment of basophils to inflammatory sites and to regulate mediator secretion. Basophils, like mast cells, express IgE receptors and are triggered for proinflammatory mediator release upon allergen:IgE binding. Both IL-3 and the newly discovered cytokine, HRF, were also found to regulate secretion. Pathways of mast cell activation apparently differ from those of basophil activation. Studies on intracellular signal transduction, a possible target for therapeutic modulation, have indicated that protein kinase C, an enzyme important in mast cell triggering, does not play a required role in IgE-dependent basophil secretion. Basophils produce a variety of mediators, including IL-4, IL-13, and MIP-1α, and the regulation of IL-4 production appears to be different from that in T cells. There is now evidence for two forms of degranulation that mediate secretion, one of which is a novel form of vesicular transport similar to that found in nerve cells. Recent *in vitro* and *in vivo* work has demonstrated that basophil FcεRI expression can be increased or decreased by as much as 100-fold, depending on factors in the microenvironment. IgE itself can mediate such changes in FcεRI expression by an as yet unknown mechanism. Thus, FcεRI is a promising target for intervention in IgE-mediated allergic responses. Intriguing recent results indicate that basophils are capable of expressing CD40 ligand and providing CD40-dependent signals, as well as IL-4, to B cells to trigger immunoglobulin class switching to IgE.

Eosinophils

In normal health, eosinophils are located principally in submucosal tissue sites, including the respiratory tract. Little is known about their mechanisms of localization or their roles in normal mucosal immunity, and the functions of eosinophils are incompletely understood even in those allergic diseases and helminthic infections that are characterized by eosinophilia in the blood and tissues. IL-5 has been shown to be highly active in the recruitment of eosinophils to airways. The role of eosinophils in allergic inflammation is considered to be associated with end-stage effector functions, based on their ability to secrete inflammatory mediators, such as the eicosanoid leukotriene C4, cationic proteins, and cytokines and chemokines, such as IL-5, IL-4, IL-6, and RANTES. It is not yet known whether selective release of mediators occurs in physiological conditions or whether release is all or none. Eosinophil effector functions are assumed to be beneficial in antihelminthic responses by killing the invading parasites, although studies in mice have not confirmed a major beneficial effect. Interactions with tissue- or extracellular matrix-derived signals that might activate eosinophils are not well understood. The expression of FcεRI and CD40 is found to be increased on eosinophils in tissues from allergic individuals.

Lipid Mediators of Allergic Inflammation: Eicosanoids and Prostanoids

The past several years have seen a growing appreciation of the complexity and sophistication of lipid mediator regulation and function in pathophysiological processes. Lipid mediators of allergic inflammation include the eicosanoids, which are produced from arachidonic acid. Arachidonic acid is released by action of phospholipase A_2 (PLA_2) enzymes during the activation-induced degradation of cell membrane phospholipids. At least seven different PLA_2 enzymes have been described, and their individual roles are not yet well understood. Once released from the cell membrane, arachidonic acid is sequentially metabolized by a series of enzymes that ultimately produce the leukotrienes LTA_4, LTB_4, and LTC_4. Arachidonic acid is also metabolized to prostanoids (prostaglandins and thromboxanes) by the action of another set of constitutive or inducible enzymes. Prostanoids include PGD_2, PGE_2, PGF_2, PGI_2, and TXA_2. It is not yet known how the supply of arachidonic acid is segregated along these different pathways, but it may involve differential enzyme localization. Several of the enzymes are located at the nuclear membrane, suggesting that these lipid mediators might function, in part, by regulating gene transcription. In support of this idea, a group of transcription factors within the steroid receptor family were found to bind certain prostaglandins. Newly synthesized prostanoids can also exit the parent cell, probably by carrier-mediated diffusion, and then act locally on the parent or neighboring cells *via* specific receptors.

Further work is clearly needed to understand the mechanisms of action in allergic situations. However, a role for leukotrienes in the pathophysiology of asthma is well established. The full clinical potential of drugs that inhibit leukotriene generation and action remains to be determined, and it is important to identify markers for the patients who are most likely to respond to antileukotriene medication. One promising area of research is likely to be the identification of single nucleotide polymorphisms within the genes of the 5-lipoxygenase/LTC_4 synthase pathway. The LTC_4 synthase is the terminal enzyme in the biosynthetic pathway for cysteinyl leukotriene production. Interestingly, the LTC_4 synthase gene is located just distal to a chromosome 5 gene cluster that is central to the Th2 response phenotype. This pathway is likely to be of great importance in asthma, in that specific polymorphisms of 5-lipoxygenase identify patients who are clinically responsive to 5-lipoxygenase inhibitors, and patients with aspirin-sensitive or intolerant asthma have an increased number of cells expressing LTC_4 synthase.

Although progress has been made in identifying and characterizing many of the enzymes involved in the generation of eicosanoids and prostanoids, much remains to be done. One of the enzymes involved in prostaglandin synthesis, called PGHS-1, is known to be regulated during development. Furthermore, its expression can be decreased in endothelial cells, and is increased in mast cells treated with stem cell factor and dexamethasone. The induction of a second enzyme, PGHS-2, is inhibited by anti-inflammatory agents such as cortisol and dexamethasone. Prostanoid receptors have been cloned and the receptor genes

have been knocked out in mice, although the specific functions of these receptors are currently unresolved. Interestingly, there is recent evidence for nuclear receptors for prostanoids. These enzymes and receptors represent potentially useful targets for pharmacological intervention in allergic inflammation, and many opportunities exist to provide needed information on their structure, function, and regulation.

Experimental Models for the Study of Allergic Inflammation

Human Models

The clear advantage of human studies is that the complexity of the microenvironment, the systemic interactions, and the unique characteristics of the relevant species for clinical applications can be maintained. The human nose, lung, skin, and eyes have served as models for allergic rhinitis, asthma, atopic dermatitis, and conjunctivitis, respectively. The large numbers of affected individuals combined with the reversible nature of the diseases have provided a large pool of volunteers for the study of onset, progression, and resolution of allergic reactions. Methods to quantify responses to antigen challenge have expanded exponentially in the past two decades and now include surface fluid assessment for changes in cell types and numbers and soluble mediator concentrations; biopsies for evaluation of anatomical structures, cell types, proteins, and RNA expression; and blood and urine analyses to identify systemic changes resulting from localized allergic reactions. Many hypotheses that were generated from *in vitro* studies have now been confirmed, and new hypotheses have arisen. Pharmacological agents with known properties have been used as probes to test the roles of different factors in the pathophysiology of the disease. Human models are of critical importance in testing the efficacy and mechanisms of action of many of the biotechnology products currently under development, such as mutant IL-4, recombinant IL-12, and peptides directed to the IgE receptor. Further development of such models will greatly facilitate the translation of basic research into clinical application.

Animal Models

In general, the sequential pathways that result in IgE-mediated mast cell degranulation, upregulation of adhesion molecules, production of inflammatory cytokines, and infiltration of leukocytes appear to be conserved between humans and animals. Various aspects of allergic responses in the lung, skin, and gut have been examined in animal models to study both IgE-mediated mast cell degranulation and the late phase lymphocyte-mediated response. Models of airway hyperreactivity have been developed to study asthma and gut resection models to study food allergies. The most widely utilized animals are mice, rats, and guinea pigs that are sensitized with real-life allergens such as house dust mite or ovalbumin, with Th2-inducing parasite antigens, or with pharmacological agents that stimulate immediate hypersensitivity reactions.

Animal models have established the role of mast cells in immediate hypersensitivity and the role of immune-specific cytokines in many allergic responses. However, one disadvantage of rodent systems is that the circulating population of leukocytes is different from that in

Research Opportunities

Genetics of Allergic Responses

- Conduct rigorous epidemiologic studies of the atopic diseases; perform targeted molecular genetic analysis to localize and identify susceptibility genes
- Define allelic variants of genes involved in the regulation of IgE production and activity, including promoter/enhancer regions and coding regions
- Examine the genetic interrelationships between atopy and other immunologic responses, such as autoimmunity and responses to parasites

Allergen Presentation to the Immune System

- Identify environmental antigens, including those in the workplace, that are responsible for inducing allergic responses in humans
- Define the MHC class II binding motifs of allergen peptides and develop analog compounds that might inhibit allergen binding
- Characterize dendritic cell differentiation pathways and APC costimulatory molecules critical for the generation of allergic T cell responses
- Identify qualitative differences in the antigen-presenting functions of different APC types that result in dominant Th2, IgE, and mucosal inflammatory responses
- Determine whether APC function can be modulated to inhibit allergic responses with cytokines or other soluble mediators
- Determine the influences of environmental factors on APC functions at mucosal surfaces

T Cell Responses

- Determine the mechanisms that control Th1 *versus* Th2 development following allergen exposure; determine whether differential migration/recruitment of Th1 *versus* Th2 cells to sites of allergic inflammation occurs
- Identify mechanisms that downregulate Th2 responses for development of immunomodulatory therapies for allergy
- Design clinical trials to test Th1/Th2-modifying protocols in atopic individuals
- Determine the role of CD8 T cells in the regulation of allergic responses

B Cell Responses

- Define the mechanisms responsible for immunoglobulin switching to the ε locus for IgE production
- Identify potential functional differences among the alternately spliced forms of membrane and secreted human IgE
- Characterize the factors that control IgE B cell trafficking and lifespan

Mast Cell, Basophil, and Eosinophil Responses

- Characterize the factors that regulate mast cell, basophil, and eosinophil development, phenotype, activation, and suppression
- Investigate the beneficial roles of mast cells in both innate and adaptive immune responses; characterize their immunomodulatory effects
- Analyze human mast cells *in situ*, *ex vivo*, and as long-term cultures
- Develop facilities to obtain highly purified human basophils in large numbers from well-characterized donors
- Identify differences in relevant signal transduction mechanisms and mediator secretion in human basophils *versus* mast cells
- Investigate regulation of FcεRI expression as an approach to downmodulate allergic reactions
- Identify the role of eosinophils in normal mucosal immune responses
- Identify eosinophil chemoattractants and eosinophil:vascular interactions
- Determine whether eosinophils can function as antigen-presenting cells to activate T cells

Lipid Mediators

- Complete the identification of enzymes that synthesize lipid mediators and the receptors that initiate their biological functions; identify redundancies in enzyme or receptor expression
- Analyze the three-dimensional structures of key enzymes and receptors to identify promising targets for therapeutic intervention
- Characterize the intracellular sites of action of lipid mediators and their roles in gene regulation
- Define the mechanisms by which anti-inflammatory compounds inhibit lipid mediator expression or function
- Determine the effects of combinations of antigen-specific vaccines and broad-acting approaches directed against components of allergic inflammation such as IgE or IL-4

humans. In the normal rodent, there is a significant pool of circulating eosinophils that is not seen in the human. The eosinophil may be a major effector cell in acute inflammatory responses in guinea pigs, a role thought to be played by neutrophils in humans. Unfortunately, only a limited number of reagents are available for the study of rats and guinea pigs, and there is a paucity of inbred strains for genetic investigation. Mouse models of allergic airway inflammation have been developed in recent years, using sensitization protocols relevant to human disease. There is an extensive array of reagents for the study of mouse cells and proteins.

Furthermore, many inbred mouse strains exist, and transgenic and gene knockout lines can be created readily. Mouse models have been used to identify or verify the importance of multiple adhesion molecules, cytokines, chemokines, and cell types involved in allergic responses. Although there are discrepancies between any one animal model and human disease, there are compelling reasons to use animal models to identify human disease pathways and investigate the underlying mechanisms and regulatory events that induce and control allergic responses. They will also prove valuable for testing protocols designed to induce allergen-specific tolerance or desensitization.

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Asthma

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Overview

Asthma is a chronic disease of the lung that is characterized by inflammation, obstruction of the airway, and increased airway responsiveness to various stimuli. The onset of asthma can occur in childhood or adulthood; in the majority of cases it first appears in childhood. The cellular infiltrates and inflammatory mediators are thought to be similar to those of other allergic diseases, but in asthma the mediators also cause airway hyperreactivity. Asthma affects more than 14 million Americans. Although all ages and races are affected, asthma deaths are 3.5 times greater among African Americans than white Americans. Both asthma prevalence and mortality have increased since the late 1970s. Asthma prevalence has doubled in westernized countries over the past 20 years, and increased by 40 percent among U.S. children during the 1980s. Asthma now accounts for about one-third of all U.S. pediatric emergency room visits. Although death is infrequent, the U.S. asthma mortality rate increased by 46 percent from the 1980s to the present, with nearly 5,000 deaths. The reasons for these increases are poorly understood.

The principal causes of asthmatic episodes are exposure to allergens or air pollutants and irritants, exercise, respiratory viral infections, withdrawal of medication, or breathing cold air. There is great variability among individuals with respect to susceptibility to these agents, as well as the severity and frequency of attacks. Asthmatic episodes are generally treated effectively with bronchodilator drugs, principally beta-2 adrenergic receptor antagonists, which are available in short-, intermediate-, and long-acting forms. Several agents are used to suppress chronic airway inflammation. The most important are glucocorticosteroids, which are the most potent agents available. Disodium cromoglycate, nedocromyl, and antileukotriene agents, which inhibit the binding of a leukotriene to its receptor or interfere with its biosynthesis, may have some anti-inflammatory properties. The introduction during the past 10 years of inhaled corticosteroid therapy was a major advance because, compared with oral dosing, it produces therapeutic effects with greatly reduced levels of systemically absorbed corticosteroids. Inhalation therapy has substantially reduced the serious side effects often associated with the long-term use of corticosteroids. Major questions remain unanswered. A hereditary pattern of asthma incidence is recognized, but the genetics of the disease remain incompletely defined. The mechanisms by which specific inflammatory cells initiate and perpetuate the asthmatic process in susceptible individuals and the immunological factors underlying exacerbations of the disease also remain incompletely understood.

Models of Airway Inflammation and Remodeling

Human and Animal Research

The experimental study of asthma in humans principally involves allergen challenge of skin, nose, or lung and analysis of biopsy specimens or bronchial-alveolar lavage (BAL) cells and fluids. Recent studies have demonstrated that asthmatics have chronic inflammation and impressive fibrosis or remodeling of their airways, indicating that chronic asthma causes irreversible changes in the lung. One striking physiological effect of allergen challenge is an increase in bronchial hyperreactivity to histamine and cholinergic agents such as methacholine. The relationship of inflammation to hyperreactivity is not fully understood. Prior investigations have demonstrated the importance of activated eosinophils, Th2-type lymphocytes, basophils, and mast cells in the inflammatory response. Unfortunately, it is not known which mediators are responsible for particular abnormalities, what contributions these mediators make singly or in combination, or which specific features of airway inflammation and/or remodeling are responsible for the physiological abnormalities characteristic of the asthmatic diathesis. From a therapeutic standpoint, treatments employing conventional immunotherapy have not been successful thus far in treating or ameliorating human asthma. Numerous animal models that utilize both allergens and nonspecific irritants have been helpful in describing airway remodeling. Animal models using induced viral infections or antigens such as ovalbumin, house dust mite, or schistosomal soluble egg antigen replicate many features of human asthma in rodents. In addition, a subset of patients who die rapidly after the onset of severe asthmatic bronchospasm apparently have unique lung pathology, in that the major inflammatory cell is the neutrophil rather than the eosinophil. The relationship of these patients to the more common eosinphil-rich inflammation of chronic asthma is unknown.

It is likely that smooth muscle function in the airway is impaired in asthma. Augmented shortening velocity corresponding to increased myosin light chain phosphorylation has been demonstrated in tissues from immune-sensitized animals, and the increased rate of shortening in muscular contraction is related to augmented maximal shortening and airway hyperresponsiveness. Although important information has been gleaned from animal models, such models have significant limitations. However, recent advances in gene overexpression, gene knockout, and dominant negative transgenic mouse modeling are very applicable to studies of airway inflammation and remodeling. This approach for asthma is still quite preliminary, and future studies should focus in detail on the interface between inflammation and myocyte function. Important advances will result from applying new methods of studying contraction in cultured cells, acutely dissociated individual airway myocytes, and myocytes from transgenic animals after specific interventions.

Mucosal Immunity and Lung Epithelium

Pathological and *in vitro* observations indicate that the cellular and structural elements of the mucosa and submucosa (epithelial cells, sensory nerves, fibroblasts, myofibroblasts, and matrix pro-

teins) interact with inflammatory cells once they are attracted to conducting airways. Nevertheless, the exact role of the mucosal immune system and epithelial function is unknown. One important observation implicating mucosal mechanisms in asthma is the characteristically thickened basement membrane. In animal models, allergic inflammation lowers action potentials of sensory nerve endings and augments parasympathetic transmission. To date, only corticosteroids have been shown to influence this mucosal immunopathology. Inhaled corticosteroids restore epithelial integrity; however, there is no evidence that basement membrane thickening can be reversed once it has occurred. Many prior studies focused on a "snapshot" approach of establishing the presence or absence of a particular spasmogen, cytokine, or histological change without testing specific hypotheses. In some cases, correlation has been presumed to be causation, resulting in misdirection and incorrect conclusions. These approaches, therefore, have not substantially increased our knowledge of the pathophysiology of asthma. There is a need to develop a detailed characterization of asthma phenotypes that correspond physiologically to the nature of airway obstruction. The ability of anti-inflammatory therapies to affect progression of asthma in these phenotypes is of particular importance, and immunohistochemical studies performed in conjunction with specific therapies would allow for assessment of specific mucosal and epithelial pathology.

The Pathogenesis of Asthma

Respiratory Infections and Other Environmental Factors

Viral respiratory infections can have major effects on asthma. First, there is increasing evidence that viral respiratory infections in early life with agents such as respiratory syncytial virus are a major factor in the development of asthma. Second, in those individuals with existing asthma, the common cold virus, rhinovirus, has been established as the major cause of acute asthma exacerbations. Thus, the interaction of respiratory viruses and infections with the atopic host is emerging as a critical factor in the development of asthma and in the regulation of its severity. Other common childhood respiratory pathogens, especially *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*, have also been implicated in asthma.

Other environmental factors have been implicated in both the pathogenesis and exacerbation of asthma. These may be separated into two groups—those environmental factors that are putative causes of asthma in individuals who are "genetically at risk," as indicated by familial susceptibility, and those environmental factors that precipitate bronchoconstriction in patients already affected with asthma. Aeroallergens, respiratory infections, passive and active exposure to cigarette smoke, very high level irritant exposure, and occupational allergen exposure have been associated with the *de novo* development of asthma. These same factors also serve to subsequently precipitate acute asthmatic events. This understanding of asthma has not uniformly led to effective preventive strategies. In addition, food

allergen exposure may be a risk factor in early childhood, since allergic children develop IgE antibodies to food before they develop IgE antibodies to aeroallergens. Trials to test the avoidance of both food allergens and aeroallergens in infancy have demonstrated variable effects in delaying the onset of allergic diseases and asthma. Double-blind, placebo-controlled studies generally are not applicable tools for the evaluation of environmental exposures, as such exposures can neither be randomized nor blinded.

Cross-sectional studies of environmental factors also have important limitations. Characterizing previous environmental exposures is almost impossible unless reliable industrial hygiene data or biological markers of infection are available. Cause and effect are difficult to differentiate in correlative studies. Case-referent studies of asthma also are limited by the requirement to characterize the environmental exposure. There are, nonetheless, promising approaches. For example, morbidity due to asthma is disproportionately high among inner-city residents for reasons that are not fully understood. Investigators supported through a National Institute of Allergy and Infectious Diseases national study on inner-city children have now shown that the combination of allergy to cockroach and exposure to cockroach allergens is an important risk factor for severe asthma among inner-city children in the eastern and midwestern United States. Thirty-seven percent of the children tested were allergic to cockroaches, 35 percent to dust mites, and 23 percent to cats. Thus, reducing exposure to the allergen as part of a multifaceted approach to asthma management may be a cost-effective method to reduce disease burden in this group. In summary, environmental exposures are important causative and precipitant factors of asthma; more research is needed to characterize more completely these factors, to elucidate the mechanism(s) by which they transform normal lungs into asthmatic lungs, and to evaluate efficacy of preventive environmental strategies.

Idiopathic Inflammatory Mechanisms

A substantial challenge of future asthma research is to identify the mechanisms by which chronic inflammation can be initiated under controlled circumstances in animals to identify agents that precipitate asthma. It is unlikely that any single animal model will succeed in guiding an understanding of the processes involved in idiopathic inflammation. Approaches that have targeted a single inflammatory cascade as necessary and sufficient to produce inflammation have been misleading in the past, but some areas worth targeting can now be specified. Examples of promising areas of study include phospholipase induction, synthesis of 5-lipoxygenase products, production of oxidants and free radicals, endothelins, and Th2-associated cytokines and chemokines. Little is known about the phenotypic changes that occur in the airway epithelia of asthmatics or how these changes predispose to and/or initiate the essential components of the idiopathic inflammatory process. Furthermore, little is known about the stimuli that induce permanent changes in smooth muscle and mucus secretion. In idiopathic forms of asthma, the infiltrating T cell appears to participate as a proinflammatory leukocyte and to possess the ability to

initiate the inflammatory response that causes asthmatic airway responsiveness.

The Genetics of Asthma

The search for genes that influence asthma-associated phenotypes is a daunting task because asthma is a heterogeneous disorder having a multifactorial nature. It has been difficult to define asthma precisely by clinical or physiological criteria, and no biochemical markers exist. These features complicate genetic studies in which phenotypes must be described as "affected" or "unaffected." Analytical approaches that circumvent these limitations to map asthma genes require large sample sizes and/or very dense genetic marker maps. There are two basic approaches to identify asthma genes. The candidate gene approach attempts to link a specific gene to a known function or phenotype. While these studies have been somewhat successful, findings have been difficult to reproduce, and it is not possible to assess the relative magnitudes of the effect. The second approach is a genomewide search, although genes with small effects are not likely to be identified. Two genomewide screens have been completed and represent the first step in identifying genes that influence asthma-associated phenotypes. One study analyzed families in western Australia and identified six genetic loci associated with markers of asthma, such as IgE responses and bronchial hyperreactivity. The second study analyzed Caucasians, African Americans, and Hispanics in the United States and identified 11 genetic loci, including 2 new loci for each of the 3 ethnic groups and 5 previously known loci. At least 3 loci in the first study are different from these 11. In addition, results in mice identified a new candidate gene for one of these loci, a gene for IL-12 responsiveness that determines Th1/Th2 development. Thus far, then, variants of at least 14 different genes have been linked to asthma phenotypes, and some of these loci differ between ethnic groups. These studies confirm previous impressions that asthma is a genetically heterogeneous disorder with many genes underlying susceptibility. The next step is to demonstrate that a particular gene in the linked regions is *the* gene, a step that has yet to be achieved for any complex phenotype. Continued mapping studies should help define subgroups of patients, identify individuals at risk for disease development, and identify mechanisms of pathogenesis (also see Chapter 15).

Innovative Approaches for the Control and Prevention of Asthma

Immunomodulation

During the past 5 years, evidence derived from biopsy and BAL samples has demonstrated that Th2 lymphocytes are selectively, uniquely, and persistently enhanced in the airways of allergic and nonallergic asthmatics. This finding and the demonstration of specific functions of the Th2 cell cytokines, IL-4, IL-5, and IL-10, have resulted in new directions for study and the potential pharmacological control of asthma. IL-4 upregulates the endothelial adhesion molecule VCAM-I, allowing for selective recruitment of leukocytes such as eosinophils, basophils, and lymphocytes that express the ligands for VCAM-I. IL-4 also promotes B cell Ig class switching to production of IgE, and IL-5 is a uniquely selective growth factor for eosinophils. IL-10 activates alveolar

macrophages and promotes Th2 cell proliferation by downregulating Th1 lymphocyte proliferation. Based on the potential importance of Th2 lymphocytes, many novel therapeutic approaches have focused on modulation of T cell activation. However, all currently available therapy causes global T cell suppression. Discontinuation of corticosteroids leads to recurrence of symptoms, and the underlying Th2 cell dominance may remain and progress. New immunomodulatory therapies should focus on the selective downregulation of the Th2 pathway. Several such approaches are now in clinical trials, including the administration of antibodies that react with IL-5, IgE, and VCAM-I. All of these approaches have shown efficacy in animal models. It should be noted that suppression of Th2 responses may lead to dominance of Th1 responses, and it is possible that Th1 cytokines may not be benign, but rather produce different pathology that is still harmful to the individual.

Lung mast cells and infiltrating eosinophils and basophils produce leukotrienes that are thought to play a role in submucosal edema and bronchial constriction. Leukotrienes are metabolites of arachidonic acid whose production is modulated by cytokines (also see Chapter 6). Protease enzymes and vasoactive peptides are also produced during asthmatic inflammation and can cause bronchoconstriction, offering additional targets for therapeutic drug development. The high-affinity IgE receptor, called FcεRI, is present on mast cells and basophils and is of central importance in asthma and allergic diseases, because allergens bind to specific IgE antibodies and IgE in turn binds to FcεRI to transduce signals for the release of histamine and other inflammatory mediators from mast cells and basophils. Recently, intravenous infusion of an antibody reactive with human IgE was shown to decrease plasma IgE levels and basophil FcεRI expression by 100-fold. Importantly, asthmatic patients treated with the anti-IgE antibody had substantial attenuation of both early and late allergic responses to antigen. Thus, FcεRI is a potentially important target for immunotherapy.

Hematopoietic Interventions: Eosinophilopoiesis

It is generally accepted that the eosinophil is the unique inflammatory cell in chronic asthma. The genesis and maintenance of eosinophilic inflammation in asthma involve several steps, including enhanced generation of eosinophils from hematopoietic stem cells (eosinophilopoiesis), their directed migration to conducting airways, and enhanced survival of eosinophils in pulmonary tissues. Animal models have demonstrated reduction of BAL and tissue eosinophils after treatment with antibody directed against IL-5. This treatment also reduces the efflux of eosinophils from bone marrow. Additional detailed studies of myeloid progenitor responses to hematopoietic influences are now needed. Other promising avenues of investigation have focused on the C-C chemokines, MIP-lα and RANTES, and the newly discovered chemoattractant, eotaxin, which may be the most specific eosinophil chemoattractant. These proteins mediate inflammatory cell chemotaxis. Investigations are needed to examine the effects of less specific inhibitory cytokines, including the antihematopoietic effects of TGFβ, which blocks myelogenesis, and to define the

Research Opportunities

Genetics of Asthma

- Identify genetic factors that contribute to abnormal mucosal immunity
- Prioritize the genetic loci that merit further study by more fully characterizing linkages identified in the asthma genome screen; organize cooperative efforts among investigators with samples from asthmatic families
- Develop improved, cost-effective methods for screening candidate genes
- Determine whether different asthma phenotypes represent different expression of the same primary genetic defect or aberrations in different genetic loci

Mechanisms of Asthma Pathogenesis

- Determine the mechanisms of action of those drugs that have proven effective in the treatment of asthma
- Characterize the cell:cell interactions and the soluble mediators that are responsible for airway remodeling; develop animal models of airway remodeling caused by chronic inflammation
- Further define myocyte dysfunction caused by asthmatic inflammation; determine how inflammation causes airway smooth muscle responsiveness and mucus hypersecretion
- Determine the mechanisms for collagen deposition in airways; evaluate the role of growth factors produced by inflammatory and epithelial cells
- Identify mechanisms by which endogenous and exogenous glucocorticoids modulate mucosal inflammation
- Evaluate the role of sensory nerves in mediating airway hyperreactivity through central and peripheral airways
- Expand studies on T cell subset biology and on eosinophil generation from bone marrow to define mechanisms of localization and chemoattraction of inflammatory cells
- Determine the mechanisms by which T cells are activated to proliferate in the lung and escape programmed cell death; investigate T cell:epithelial cell interactions and epithelial-derived cytokines

Environmental Stimuli

- Develop methods for exploring the roles of viruses and environmental antigens in generating airway inflammation and remodeling in both infants and adults
- Determine the effects of various respiratory infections on Th1 and Th2 cytokine profiles
- Determine the relationship between the age of respiratory infection and the development of asthma or its prevention
- Establish animal models, including transgenic and gene knockout models, to determine the effects of respiratory infections on lung function, lung growth and development, and allergic inflammatory responses
- Develop antiviral therapies to prevent virus-induced asthma

Asthma Therapy

- Identify gene promoters that can be used in a transgenic system to target potentially therapeutic proteins to airway myocytes, non-Clara epithelial cells, or dendritic cells
- Develop methods to regulate gene transcription externally to generate targeted airway-specific gene disruption
- Identify opportunities to relate findings in animal model systems to human materials from asthmatic subjects
- Ascertain whether early intervention by anti-inflammatory or immunomodulating agents prevents the onset of persistent asthma
- Determine whether early avoidance of allergens correlates with decreased asthma incidence or severity
- Correlate human asthma phenotypes with the future course of the disease

Immunomodulation

- Define the roles of IL-4, IL-5, and IL-10 in the pathogenesis of asthma
- Determine whether the Th1/Th2 imbalance seen in asthma is caused by defective inhibition of Th2 development/activity, by enhanced Th2 development/activity, or by defective Th1 development/activity
- Investigate the pathogenic potential of Th1-dominant responses to allergens or environmental stimuli
- Evaluate the role of cytokines and chemokines in inflammatory cell proliferation and migration/survival in the lung airways

mechanisms of eosinophil apoptosis and the relationship of apoptosis inhibition to the pathogenesis of asthma. The role of Fas ligand in this process is of particular interest, and the mechanisms of inflammatory cell death remain a fertile area for additional research.

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Autoimmunity

8

Overview

Autoimmunity occurs when the normal immune discrimination between self and nonself is altered and the individual begins to respond to his or her own tissues, cellular constituents, or proteins. Although autoimmunity is now known to be common, it usually causes minimal tissue pathology. In many circumstances, tissue or organ damage can initiate the production of immune responses to self, but these infrequently contribute to ongoing tissue injury. Only when self-reactivity causes cellular, tissue, or organ damage can it be said that the disease is autoimmune in etiology. After the initial recognition of autoimmunity as a cause of tissue pathology in humans about 50 years ago, the major focus was on descriptions of the spectrum of autoantibodies, specific disease associations with autoantibodies, and details of the pathogenic consequences of autoimmune responses. In 1973, the first association of a suspected autoimmune disease, ankylosing spondylitis, and a specific gene product, namely HLA-B27 encoded by the MHC gene complex, was described. Since then a variety of associations of specific MHC genes with other autoimmune diseases, including rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, and insulin-dependent diabetes mellitus, have been identified.

A general hypothesis to describe the pathogenesis of autoimmune diseases has emerged, in which an unknown environmental trigger is thought to generate abnormal immune responses against self components in genetically susceptible hosts. Although unproved, this hypothesis is widely accepted. Further research is needed to define more comprehensive genetic associations with disease, to determine the biochemical basis for such associations, to identify the specific triggers of disease initiation, and to develop and apply therapeutic approaches to halt the progress of disease. It is important to note that autoimmune diseases can affect essentially every organ in the body. There are systemic autoimmune diseases, like systemic lupus erythematosus (SLE), in which many self antigens are targets for autoantibodies and many organs are subject to autoimmune tissue damage. There are also organ-specific autoimmune diseases in which autoreactivity and tissue damage are limited to a single organ. Whether the same mechanisms give rise to both types of autoimmune disease and whether the same therapeutic interventions will be beneficial is not known. Current therapies of many autoimmune diseases rely on global immunosuppression with corticosteroids and cytotoxic drugs. This approach does not distinguish autoreactivity from protective immune responses and so leaves the patient at risk for serious infection. A major goal of continued research into autoimmunity is to devise new therapies that will diminish pathogenic autoreactivity or even prevent it in individuals known to have susceptibility factors, without diminishing protective immunity.

Genetics of Autoimmunity

Most autoimmune diseases appear to have multiple susceptibility genes. In animal studies, backcross analyses as well as the generation of recombinant inbred mouse strains have led to the identification of chromosomal loci that appear to contain susceptibility genes. Recombinant inbred mice are families of genetically defined mice that differ in only one or a few loci. These strains of mice have been particularly important in associating specific autoimmune phenotypes with particular chromosomal regions, and their continued use, together with transgenic and gene knockout mice, should permit investigators to dissect the multiple components of autoimmune disease in finer detail and begin to understand the functions of homologous genes in human disease pathogenesis.

Initial animal studies have indicated that the induction of autoimmunity and subsequent damage to target organs are regulated by different genetic loci. Furthermore, there appear to be certain susceptibility loci that may be common to many autoimmune diseases, whereas others appear to be unique to a particular disease. These observations open new opportunities to consider preventive interventions. An additional important observation is the finding that there are resistance genes to autoimmune disease as well as susceptibility genes. Understanding the nature and function of these resistance genes should also suggest new opportunities for therapeutic interventions.

Genetic studies of autoimmune disease have begun in humans and have led to the identification of chromosomal regions that harbor susceptibility genes. Many of these regions are in the same genetic locations as regions associated with disease susceptibility in the mouse, indicating that mice will serve as useful models for understanding human disease. In human studies, a recent focus has been to determine whether racially diverse populations have the same susceptibility/resistance genes, because disease incidence is often skewed to particular populations. For example, SLE is approximately three times more common in African Americans and Hispanics than in those of northern European ancestry. Some investigators have identified a genetic locus that they believe correlates with lupus nephritis in African Americans, although others believe this locus contains a susceptibility allele for all populations. Similarly, a set of MHC genes is highly associated with rheumatoid arthritis in northern Europeans, but not in African Americans or Hispanics. Many autoimmune diseases, such as rheumatoid arthritis and SLE, are much more common among women than men, although a few, such as ankylosing spondylitis, show the opposite bias. The basis for gender impact is not well understood, and expanded research efforts are needed in this area.

Mechanisms of Peripheral T Cell Tolerance and Autoimmunity

In the past 10 years there has been a surge of interest in analyzing the mechanisms of immunological tolerance in mature peripheral lymphocytes and the importance of these mechanisms in preventing autoimmunity. There are two major reasons for this recent interest: (1) the realization that tolerance to many

self antigens is maintained in the periphery and not by deletion of immature autoreactive lymphocytes in the generative lymphoid organs, and (2) the discovery of mutations that cause autoimmunity by interfering with peripheral tolerance. Two technological advances have been especially important in furthering progress in this area: (l) the use of antigen receptor transgenic mice as models to study immunological tolerance and autoimmunity and (2) the creation of gene knockout animals that more closely mimic human disease by developing "spontaneous" autoimmune disease, without the overt immunization with self antigen that is usually required in many intact animal models. Recent work from a number of laboratories has begun to define the mechanisms that normally regulate self-responsive mature lymphocytes (also see Chapter 9). In general, peripheral tolerance is induced by (1) self antigen presentation to the immune system in a context that favors deletion or anergy rather than lymphocyte activation or (2) regulatory lymphocytes that inhibit effector cell responses to self antigens.

Repeated antigenic stimulation of T lymphocytes, particularly CD4 helper cells, leads to surface expression of the apoptosis-inducing molecule, Fas, and its ligand, FasL. Engagement of Fas by FasL triggers apoptosis, resulting in activation-induced cell death of both T and B cells. The importance of this death pathway in the maintenance of self-tolerance was dramatically illustrated by the discovery that active disease in two mouse models of lupus-like systemic autoimmunity, the *lpr/lpr* and *gld/gld* homozygous strains, is caused by defective expression of Fas or a point mutation in FasL, respectively. The available evidence indicates that the Fas pathway is normally involved in the activation-induced death of mature CD4 T cells and B cells in peripheral lymphoid tissues and helps maintain homeostasis and self-tolerance. Importantly, Fas mutations have been discovered in children with autoimmune disease and lymphadenopathy, thus providing an example of results from animal studies guiding a successful search for the etiology of human disease. It is becoming clear, however, that Fas/FasL abnormalities are not the basis for human SLE, although blocks in Fas-mediated apoptosis may play a role in the synovial proliferation characteristic of rheumatoid arthritis. Interestingly, autoimmune diseases develop in mice in which the genes for IL-2 or the IL-2 receptor α or β chain are knocked out, and this result may be related to failure of Fas-mediated cell death. The role of IL-2 in potentiating the Fas death pathway was first described over 5 years ago, but its importance is only now being demonstrated. Recent animal data indicate that intact IL-2/IL-2 receptor signaling pathways are required for the optimal expression of the Fas ligand gene product. These results raise the possibility that defects in IL-2 production and/or signaling may contribute to systemic autoimmune diseases in humans. Other recent studies show that autoimmune disease develops even when a deficient Fas molecule is present only in the B cell compartment, demonstrating the critical role Fas plays in B cell regulation. Whether IL-2 is also involved in potentiating this pathway is not known.

In addition to cell death, T cell anergy (functional inactivation without deletion) may result in peripheral immune tolerance. A widely held view is that anergy results from T or B cell recognition of

antigen in the absence of signals provided by costimulators. For T cells, recent results further indicate that antigen recognition in the presence of costimulation can result in anergy if the inhibitory T cell receptor CTLA-4 is also engaged. These results suggest an explanation for the fatal autoimmune disease that develops in CTLA-4 knockout mice. The role of CTLA-4 abnormalities in human autoimmune disease is an area of active investigation. For B cell activation, the interaction of CD40 on the B cell with CD40L on the T cell is critical. Interruption of this signaling pathway will prevent B cell activation, and overexpression of CD40 or CD40L appears to favor autoreactivity.

Therapies of autoimmune disease based on inducing peripheral tolerance by blocking costimulatory molecules are being actively investigated in a number of animal models as well as in human disease. Thus, recent results confirm the importance of cell death and functional anergy in the maintenance of peripheral self-tolerance and the roles of various cell surface and secreted molecules that might serve as targets for pharmacological manipulation of these processes. However, much remains to be learned about the nature of self-tolerance and the factors that influence the balance between cell death, anergy, and pathological activation.

Microbial and Environmental Triggers for Autoimmune Disease

Studies of environmental factors have focused largely on the concept that microbial exposure may initiate some aspects of autoimmune disease. Numerous studies have suggested that mice prone to develop autoimmune disease do so more aggressively when exposed to microbial organisms. Moreover, some autoantibodies and T cells derived from patients with autoimmune disease have been shown to cross-react with self and various microbial antigens. For example, in diabetes it was shown that T cells recognizing a peptide of the self antigen, glutamic acid decarboxylase, will also be activated by a peptide derived from a Coxsackie B virus protein, thus raising the possibility that Coxsackie B infections might be the trigger for a very characteristic autospecificity in diabetes. Similarly, T cells from individuals with multiple sclerosis will cross-react with both the self antigen, myelin basic protein, and a measles virus protein. Antibodies may also display cross-reactivity between self and foreign antigens. In SLE, anti-DNA antibodies have been shown to cross-react with bacterial polysaccharide. Findings such as these have stimulated a reexamination of the role of environmental factors in human autoimmune disease. Of note, analysis of this issue has suggested the existence of additional, hitherto unrecognized, autoimmune manifestations. For example, the late sequelae of Lyme disease can include the development of arthritis, which may involve the induction of an autoimmune process.

Microbes and other environmental agents might elicit autoimmune disease by a variety of mechanisms. It is clear that microbes might function as adjuvants by activating antigen-presenting cells and eliciting cytokines that enhance immune responses to self-antigens in susceptible

hosts. It is known that autoreactive T and B cells are not completely eliminated by self-tolerance mechanisms even in healthy individuals. These cells do not usually become pathogenic, perhaps because they have low-affinity antigen receptors, because the self antigen is present in low concentrations, because costimulation is not available, or because other conditions preclude activation. However, homeostatic disruptions such as inflammation following infection might produce appropriate conditions for activation of these self-reactive lymphocytes. Evidence for this mechanism of induction of autoimmune disease comes from studies of lupus-prone mice, rodents expressing the human HLA-B27 molecule, and animals with genetically altered T cell repertoires enriched in autoreactive T cells. In these animals, exposure to environmental antigen or to specific bacteria or bacterial products can exacerbate autoimmune disease, often because the microbe has adjuvant activity. An additional mechanism by which microbes might elicit autoimmunity could be direct T cell stimulation by microbe-derived superantigens, which are antigens that have the unusual property of stimulating very large numbers of T or B cells, some of which may have self antigen-reactive receptors. These models offer exciting opportunities to study the triggers of autoimmunity in genetically predisposed hosts.

Another model that was developed in greater detail recently is one in which molecular mimicry between microbial and self antigens induces autoimmune disease. Perhaps the clearest example of this is rheumatic fever, a disease that is precipitated by streptococcal infection. Antibodies reactive with the streptococcal M protein also bind to cardiac myosin heavy chain, and these antibodies are implicated in the carditis of rheumatic fever. In some cases, individual T cell clones or antibodies from patients or animals with autoimmune disease have been shown to recognize both self and foreign peptides, the phenomenon called molecular mimicry. Thus, a strong infectious stimulus might activate normally quiescent lymphocytes that then can respond to the self antigens that cross-react with the pathogen. The identification of target autoantigens may therefore provide clues to the etiology of autoimmune disease.

Another potential role for infection in the breakdown of self-tolerance is the activation of lymphoid cells by cryptic epitopes of self antigens. This model assumes that pathogenic autoreactivity is directed to cryptic self epitopes that are usually either presented at a very low level by MHC molecules, a level below the threshold needed for tolerance induction or activation, or are not processed and presented at all. Autoreactivity then might arise when the density of such peptide-MHC complexes is increased in the presence of costimulatory molecules induced by infection or when antigen processing is altered by infection to yield epitopes that are not usually presented. For example, it appears that B cells may frequently present peptides of self antigens that are not presented by macrophages or dendritic cells, the cells involved in thymic tolerance induction. In this model, infection elicits autoreactivity by promoting the presentation of increased numbers of self epitopes following tissue damage. Autoreactivity might also result from B cell presentation of altered self epitopes containing regions of similarity shared by

self and foreign antigen. Subsequently, anti-self B cells would ingest, process, and present epitopes derived from additional parts of the initiating molecule, not originally recognized as immunogenic, resulting in "epitope spreading" and expanded autoreactivity. A potential role for cryptic epitopes of self antigens in a number of animal models of autoimmune disease has been indicated, as has a role for such epitopes in the initiation of human rheumatoid arthritis.

Additional studies have shown how immunization with environmental pathogens may lead to the production of specific autoantibodies. For example, immunization with the bacterial antigen, phosphorylcholine (PC), leads to the generation of B cells producing antibodies that bind both PC and double-stranded self-DNA, the major pathogenic specificity of SLE. These autoantibodies are only transiently expressed in non-autoimmune-prone mouse strains, and they can have either protective or pathogenic potential. It appears that the B cells producing these antibodies have acquired autospecificity by somatic mutation of their immunoglobulin genes and that these cells are routinely downregulated in non-autoimmune mice. Whether autoimmune disease arises in some individuals because they are unable to downregulate B cells that acquire self-specificity after antigenic stimulation and somatic mutation remains a question requiring further exploration. This area of research holds promise for understanding disease pathogenesis and for trials of new therapeutic strategies.

It is important to remain aware that the induction of a particular autoimmune disease by a particular microorganism, or other environmental antigen, may occur only in human and not in rodent models. Therefore, it is critical to perform epidemiological studies and studies of autoreactive T and B cells in humans. It is also critical to note that microorganisms are not the only potential triggers of autoimmune disease. A variety of environmental antigens have been studied for their ability to induce autoreactivity. In addition, new data implicate microchimerism, the long-term retention of viable maternal cells in offspring or of fetal cells in the mother, in the etiology of certain autoimmune diseases.

Studies of the induction of autoimmune diseases may also help in the design of cancer therapies. The recent awareness that the immune response to tumors is often a response to self antigens, together with the development of promising tumor vaccines, has led to the suggestion that induction of a destructive tissue-specific autoimmune response may be an approach to cancer therapy. For example, an autoimmune response to prostatic cells might represent a treatment for prostate cancer locally as well as for distal metastases. The relationship between autoimmunity and effective tumor surveillance is an area that promises to yield much useful information.

Animal Models of Human Autoimmune Diseases

Several animal models that have been particularly informative involve transgenic mice that express a foreign antigen as a self antigen and are subsequently

immunized with the foreign antigen. Often the mice are also transgenic for a T cell antigen receptor or antibody molecule that recognizes the foreign antigen. These studies have great potential not only for understanding disease pathogenesis but also for devising effective therapies. For example, a transgenic (tg) mouse model was developed in which a known viral antigen was expressed in the insulin-producing beta cells of the pancreatic islets of Langerhans. Because the viral antigen was integrated within the animal's germ line and passed onto progeny mice, it is a "self" antigen in all respects. Spontaneous insulin-dependent diabetes mellitus (IDDM) does not occur in these tg mice expressing the viral antigen in the pancreas (incidence <1 percent). However, even when the viral transgene is expressed in the thymus as well as the beta cells to ensure good self-tolerance, anti-self (viral) lymphocytes are found in the periphery. These cells remain nonresponsive unless IFNγ or a B7 costimulatory molecule is coexpressed as a second transgene in the beta cells. Under these circumstances, IDDM does occur. Peripheral unresponsiveness can also be broken by infection with whole virus, which leads to islet destruction and development of IDDM in more than 95 percent of the tg mice. Without thymic expression of the viral transgene, IDDM occurs within 2 weeks of infection and it correlates with the presence and activity of high-affinity cytolytic T cells. By contrast, these high-affinity T cells are deleted in mice expressing the transgene in the thymus, with only low-affinity T cells entering the periphery. With the appropriate stimulation/costimulation, however, these low-affinity T cells cause the same incidence of IDDM (>95 percent) but with slower kinetics; disease is seen at 1 to 8 months postviral challenge. The genetic background, especially the MHC type, markedly influences disease susceptibility.

Model systems such as these have generated a number of insights into the mechanisms of autoimmune tissue pathology and potential means by which environmental agents may trigger autoimmune diseases. These models are also informative in genetic studies, because disease induction or lack of maintenance of peripheral tolerance often occurs only when the transgenes are expressed in particular inbred mouse strains. Backcross analysis between disease-susceptible and disease-resistant animals can help identify susceptibility and resistance genes, as well as the multiple pathways that regulate autoreactivity. Thus, transgenic models can help elucidate the multigenic basis of autoimmune disease. Other animal models that hold great promise have genetically engineered overexpression or underexpression of molecules within intracellular signaling pathways. It is currently suspected that variations in these pathways may contribute significantly to human autoimmune disease.

Disease Pathogenesis

In some animal models of autoimmunity, there appears to be a spontaneous, age-related expression of autoreactivity, whereas in other models, specific immunizations or other manipulations are required to induce disease. In humans, disease appears to occur spontaneously, although unknown environmental triggers may well play an important role. In some models, animals recover and remain disease free thereafter; in others,

the animals proceed to a chronic relapsing and remitting disease course. Most human autoimmune disease is characterized by exacerbation and remission. Therefore, it remains critically important to study the chronic relapsing models of autoimmunity as well as models where disease progression is unremitting.

One major recent observation is that the ratio of Th1- to Th2-type cells may be critical for remission in a variety of animal models of autoimmunity. It also appears that there are other populations of regulatory cells that may be important for disease remission. For example, a population of antigen-specific T cells that secrete the cytokine TGFβ (Th3-type cells) was found to have immunosuppressive properties. There is also a population of NK 1.1 cells in the mouse, and an apparently equivalent population in humans, that may play an important role in disease regulation. Further characterization of such regulatory T cells remains a major challenge for the next several years.

It is important to understand mechanisms of target organ injury in autoimmune disease. This requires understanding which effector functions of activated cells mediate tissue damage and the response of the target tissue to cytokines and other soluble mediators of inflammation. New data suggest that there may also be genetically controlled differences in target organ susceptibility.

It is clear that a number of soluble factors not produced by lymphoid cells, such as neuropeptides and sex hormones, are also involved in setting thresholds for activation or apoptosis of lymphoid cells and that immune function alters with age. These are areas of active investigation. An understanding of the neuroendocrine-immune axis in particular may provide insights into the reasons why some diseases have a higher incidence in either men or women, why many autoimmune diseases do not develop until puberty, why pregnancy can transiently change the severity of disease, and how stress might alter disease expression.

Therapy

Animal models can be used to test new therapeutic strategies. Induction of oral tolerance is one novel therapy that has been tested in transgenic mice. As discussed above, transgenic mice expressing a viral antigen in both thymus and beta cells develop slow-onset IDDM, with an incidence of greater than 95 percent at 1 to 2 months after viral challenge. However, when insulin was given orally twice per week, starting before or even 10 days after viral infection and continued until 6 weeks postinfection, IDDM was prevented for at least 6 months in over 50 percent of the treated mice. Prevention of diabetes was associated with a reduction in the islets of lymphocytes expressing IFNγ (Th1-type cells) and an increase in the number of lymphocytes producing IL-4 (Th2-type cells). However, when one or two amino acids were mutated in the insulin molecule, no protection was observed and the onset of IDDM was actually hastened. While it is not known exactly how oral insulin might protect against diabetes, recent studies have suggested that peptide presentation and T cell activation in intestinal mucosal lymphoid tissue may induce a different T cell subset with a different pattern of cytokine

Research Opportunities

Autoimmune Disease Mechanisms

- Distinguish the mechanisms of disease induction, remission, relapse, and organ damage
- Identify the epitopes of self antigens recognized by autoreactive lymphocytes; use this information to identify cross-reactive epitopes on infectious agents that may be associated with autoimmune disease
- Define the molecular mechanisms responsible for the loss of self-tolerance to identify targets for therapeutic intervention after disease onset

New Animal Models

- Develop models of chronic relapsing and remitting disease that more closely mimic human disease; models with alterations in cell surface molecules or signaling pathways; and mice that express key human molecules as transgenes
- Use animal models to discover or test candidate genes involved in susceptibility or resistance to autoimmune disease and functions of the gene products
- Investigate the potential for cytokine modulation to treat autoimmune disease

Studies on Human Autoimmune Disease

- Identify multiple genetic loci linked to disease susceptibility or resistance, and to gender and racial associations, in humans
- Define microbial components that initiate or exacerbate destructive autoimmune responses in humans
- Develop pilot clinical trials for the prevention or reversal of human autoimmune disease

Nonlymphoid Factors That Contribute to Autoimmune Disease

- Develop a greater understanding of the neuroendocrine-immune axis and its role in disease initiation and progression
- Investigate the molecular basis for gender differences in disease incidence and severity

production, perhaps reflecting different costimulatory molecules in the gut. Alternatively, a regulatory T cell subset may be generated that prevents activation of the pathogenic T cell subset. Further studies are necessary to understand how oral administration of antigen might prevent or retard autoimmune disease. Oral insulin treatment is now being tested in humans; human trials in diabetes and other autoimmune diseases must carefully select the correct antigen molecule for therapeutic value, since the wrong treatment might enhance disease.

Other therapeutic approaches in autoimmunity include blocking the activation of cytolytic T cells by injecting mice with a synthetic "MHC class I blocking" peptide. Alternatively, T cell activation can be reduced by aborting MHC expression specifically in the islets. As discussed above, therapies that interfere with costimulatory signals were explored in animal models prior to the initiation of clinical trials in humans. Cytokine-based therapies were also pioneered in animal models and now appear to be effective in certain autoimmune diseases. Finally, it is important to acquire a better understanding of the mechanistic basis of certain existing therapies. For example, intravenous immunoglobulin appears to help in some autoimmune diseases, but its mechanism of immune modulation is not known.

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Transplantation

9

Overview

Since the inception of transplantation at the beginning of the 20th century, the principal goal has been the physical and functional replacement of failing organs and tissues. Successful clinical application of transplantation, however, had to await the development of effective methods for suppressing host immune responses against the graft, which otherwise would stand as an absolute barrier to the transplantation of all but genetically identical tissues. In the past 30 years, the development of immunosuppressive agents and the refinement of ancillary therapies have made transplantation the preferred treatment for some of the most widespread diseases that cause disability and death, including failure of the kidneys, heart, liver, lungs, hematopoietic system, and, in some circumstances, the pancreas. Furthermore, transplantation offers the potential for delivery of medical care to parts of the world where more complex technologies cannot be adopted to treat chronic diseases. For example, bone marrow transplantation is used to treat thalassemia and sickle cell disease in areas where chronic transfusion therapy is not feasible. Thus, transplantation offers a context in which immunology has a broad and profound impact on human health.

Transplantation can also be used to ameliorate the toxicity of other therapies. For example, bone marrow transplantation is currently used in the treatment of cancer to reconstitute the hematopoietic system following high-dose chemotherapy, and is being explored as an approach to induce immunological tolerance and as a vehicle for gene therapy. There is also evidence that allogeneic bone marrow transplantation provides cancer immunotherapy mediated by graft-*versus*-leukemia responses. Valuable information about normal and pathogenic immune responses has been obtained from transplantation studies, which have clarified fundamental concepts of tolerance, immunogenetics, and the central importance of the MHC in cellular immunity. Transplantation models are used to test new therapeutic agents and immunomodulatory regimens, and in turn, certain of these therapeutic agents have provided new understanding of basic cell biology, such as intracellular signaling events in T cells.

Although transplantation is among the most successful and promising of recently developed therapeutic modalities, considerable challenges still exist. One major challenge remains the immune response of the recipient against the graft, that is, rejection. Effective immunosuppressive therapy had to be developed to make transplantation possible, and introduction of the drug cyclosporin A (CsA) in the 1980s allowed 1-year survival rates for solid organ transplants to exceed 50 percent for the first time. CsA blocks the calcineurin/calmodulin signaling pathway required for T cell activation and thereby inhibits lymphokine production and proliferation of graft-reactive T cells. Furthermore, in the event of a rejection

episode, monoclonal antibodies can be used to rescue the graft, either by modulating the T cell antigen receptor (TCR) or by depleting T cells. One such reagent is the widely used antibody called OKT3®, which specifically binds to the TCR:CD3 complex that mediates activation signals. More recently developed immunosuppressive agents, such as mycophenolic acid, rapamycin, brequinar, leflunomide, and "humanized" monoclonal antibodies, promise to further reduce or even eliminate the problem of acute allograft rejection. In fact, the use of mycophenolic acid together with other agents has reduced the 1-year kidney rejection rate to less than 15 percent.

Unfortunately, all these treatments require long-term/lifetime therapy, and they nonspecifically suppress the entire immune system. This prolonged, broad-based therapy exacerbates the cumulative toxic effects of the drugs and exposes patients to considerably higher risks of infection and cancer. Moreover, early acceptance of a functioning graft is often followed in later years by a chronic rejection process that limits the life of the graft and is responsible for a high rate of retransplantation. Remarkably, one-third of those on waiting lists for a transplant are patients who have lost a prior graft. To prevent the many problems that still exist in transplantation, intense research is focused on the induction of donor organ-specific tolerance to provide, for the first time, a means of selectively inactivating only those immune cells that destroy the graft, without compromising the entire immune system. Thus, one major goal of transplant immunology research is the induction and maintenance of donor antigen-specific tolerance.

Currently, human donors are the only source of organs and tissues for human transplantation. Transplants such as these, between genetically different members of the same species, are called allogeneic, or allotransplants. One of the most daunting challenges to the full application of transplantation is the shortage of suitable human donors. In the case of solid organ transplantation, this shortage limits clinical application to as little as 5 percent of the procedures that could be performed if the supply of organs were unlimited, and results in approximately nine deaths per day among patients in the United States waiting for transplant. Such limited supply clearly precludes the use of allotransplantation early in disease or as a preventive measure and raises ethical issues and controversies about the allocation of available organs. One potential approach to the organ shortage problem is xenotransplantation, which is the transfer of animal organs or tissues into humans. In certain cases, such as viral hepatitis, xenotransplantation also may offer a means to avoid the recurrence of the original disease, and it offers a potential method for gene therapy to correct specific disorders or block immune rejection because donor animals could be genetically engineered to express specific therapeutic proteins. However, the immune barrier to xenotransplantation is even greater than that to allotransplantation. This and other considerations, such as the danger of transmitting animal viruses into humans, limit the current use of xenografts, although continued research may provide solutions to these problems. This report will summarize advances and recent developments in the field of transplantation and suggest areas in which significant research opportunities exist.

Biological Hurdles to Transplantation

T Cell Immunity and Allograft Rejection

The primary biological barrier to allotransplantation is the cellular immune response of the recipient against the genetically mismatched graft. Cellular immune responses are mediated primarily by T lymphocytes, which have antigen-specific receptors that recognize the foreign antigens of the graft. The major histocompatibility antigens (MHC; called HLA in humans) are primary targets for allorecognition, but responses directed against minor histocompatibility antigens can cause rejection even in HLA-matched allotransplants. T cell activation occurs shortly after transplantation and, in the absence of immunosuppression, leads to graft rejection within days to weeks.

Antibody-Mediated Graft Rejection

Organ and tissue allotransplants also induce the synthesis of antibodies by B cells, and such alloreactive antibodies can cause acute rejection even when cellular rejection is prevented by immunosuppressive drug therapy. For example, acute vascular rejection is thought to be mediated by antidonor antibodies. If a recipient has preformed antidonor antibodies at the time of transplantation, hyperacute, immediate rejection is likely to occur. Hyperacute rejection is caused by complement activation, which is stimulated by the binding of antidonor antibodies to antigens expressed on donor organ endothelium. Thus, organ transplantation is a very limited option if the potential recipient already has antibodies that react with donor blood group antigens or HLA molecules. A significant proportion of potential recipients do, in fact, have anti-HLA antibodies as a consequence of prior blood transfusions or prior transplants that failed. Because these antibodies cross-react with broad subsets of HLA types, finding suitable organs for these patients is a major challenge, and presensitized patients may never receive a transplant.

Chronic Graft Rejection

Control of acute rejection in clinical practice depends on (1) blood group and HLA matching, (2) use of the preoperative cross-match to avoid transplanting organs to presensitized patients, and (3) the use of nonspecific immunosuppressive drugs. This strategy now prevents immunologic destruction of transplanted organs during the first year in greater than 90 percent of patients. However, the treatments frequently fail to prevent chronic rejection, which is responsible for the loss of 3 to 5 percent of transplanted organs per year thereafter. Chronic rejection of kidney and liver transplants and the similar lesions that occur in heart (accelerated arteriosclerosis) and lung (bronchiolitis obliterans) transplants are now the major cause of allograft loss. Loss of organ transplants beyond the first year may also result from recurrence of the primary disease, autoimmune responses, or viral infections. Clearly, a major focus for future research is to identify the mechanisms of chronic rejection and other late problems and develop appropriate therapies for prevention.

Hurdles to Bone Marrow Transplantation

Bone marrow transplantation is designated as *autologous* when the recipient is his or her own donor, *syngeneic* when the donor is an identical twin, or *allogeneic*

when the donor is not genetically identical to the recipient even if they are HLA matched. As in solid organ transplantation, the major limitations of allogeneic bone marrow transplantation include donor availability, treatment-related toxicity, and graft rejection. In addition, however, the development of acute and chronic graft-*versus*-host disease (GVHD) mediated by T cells and relapse of a primary malignancy represent significant challenges to successful clinical outcome. Transplantation of autologous marrow cells generally results in engraftment without GVHD; however, the likelihood of neoplastic relapse is greater than for allogeneic marrow transplants. The use of allogeneic donor cells may reduce the chance of relapse for some neoplasms but is associated with a greater incidence of early transplant-related mortality. Furthermore, T cell depletion of allogeneic marrow to prevent GVHD has been associated with increased graft rejection and increased leukemia relapse.

HLA-matched bone marrow obtained from a sibling is considered to be the optimal source for allogeneic transplantation. However, only 20 to 25 percent of patients in need of a transplant have a readily available donor in this category. In the past two decades, the lack of genetically matched, related donors for the majority of patients has led to a large number of clinical activities to define alternative donors. Strategies include the use of T cell-depleted hematopoietic stem cell populations, unrelated matched or partially mismatched living volunteer bone marrow donors, and, most recently, banked unrelated umbilical cord/placental blood.

An additional problem in the transplantation of bone marrow and other cell types is the need for an appropriate host microenvironment to support the vitality, function, and proper regulation of the transplanted cells. It is known that some anatomical sites are insulated against immune responses, and the use of naturally "privileged" sites, or construction of artificially sequestered sites that provide an appropriate microenvironment, is an area of current research for cellular transplants.

Hurdles to Xenogeneic Solid Organ Transplantation

Xenotransplantation as an alternate means for providing organs and tissues for transplantation is not yet a clinical reality, except in early experimental trials. One of its major limitations is the human immune reaction against the animal graft. For a variety of reasons, pigs are considered to be the optimal donors for xenotransplantation. However, vascularized organs such as pig heart or kidney are subject to extremely rapid, hyperacute rejection when transplanted into humans. This response is initiated by the binding of naturally occurring, xenoreactive antibodies to the graft. The offending antibodies are predominantly IgM, and they react with the antigen Gal-alpha-1-3-Gal, a sugar molecule expressed on the endothelial cells of lower mammals. Antigen-bound IgM rapidly activates the innate complement system, which then destroys the graft. Because proteins expressed in the graft endothelium that would normally inhibit complement activity are unable to do so across species, the xenograft is especially susceptible to complement-mediated injury. This problem has recently been addressed with

very promising results by genetically engineering pigs to express human complement inhibitory proteins, such as decay accelerating factor, CD59, and membrane cofactor protein. However, once hyperacute rejection is prevented, a pig organ xenograft is still subject to immune-mediated acute vascular rejection and cellular rejection. Another hurdle to xenotransplantation is the potential threat to public health if animal viruses that currently do not infect humans become capable of human transmission following direct introduction into transplant recipients.

Current Work and Future Directions

Targeting T Cell Signaling Molecules

Two areas of intense current research are (1) improved immunosuppressive drug development and (2) the induction of specific immune tolerance. A rapidly growing body of knowledge on T cell activation has led to a change in the development of new immunomodulatory agents. In the past, discovery of effective immunosuppressive drugs depended largely on serendipity and empiric observations. For example, the first immunosuppressive agent, 6-mercaptopurine, was initially developed as an antitumor drug. Now there is a more rational approach to the development of therapeutic agents broadly based on new understandings of T cell and transplantation biology. For example, small molecules are being developed to inhibit signal tranduction or transcription factors utilized in T cell activation. Although conventional drug discovery approaches still dominate the market, new approaches developed from advances in basic immunology will broaden the repertoire of immunomodulatory agents, potentially reduce the complications of immunosuppressive therapy, or even induce immune tolerance. A new reagent that targets activated T cells is a humanized monoclonal antibody that binds to high-affinity IL-2 receptors and prevents T cell proliferation. Phase III clinical trials showed that this agent significantly reduces acute rejection of kidney grafts without added toxicity, and it is being studied in a variety of trials for effects in other transplant situations and in autoimmune disease.

Regulating T Cell Responses Through Signal 1 (TCR Ligation by MHC:Antigen)

Activation of T cells requires signaling not only through the TCR (Signal 1) but also through nonpolymorphic costimulatory molecules (Signal 2). Therapeutics designed to disrupt early TCR signal cascades have shown promise as immunosuppressive drugs. Furthermore, reagents that target Signal 1 during initial antigen recognition to induce long-term, antigen-specific tolerance in the absence of chronic drug therapy are the focus of much current research. Antigen:MHC binding to the TCR:CD3 complex initiates a cascade of signaling events beginning with activation of several cytoplasmic protein tyrosine kinases. Recruitment of the CD4 (or CD8) coreceptor and its associated tyrosine kinase, Lck, into the vicinity of the TCR complex is believed to induce phosphorylation of CD3 proteins, which then sequentially capture and activate the cytoplasmic tyrosine kinase, ZAP70, leading to downstream signal progression (see Chapter 1, Figure 1-6). The clinical importance of ZAP70 is highlighted by the recent identification of patients with selective T cell deficiencies resulting from

mutations of the ZAP70 gene. Interestingly, certain anti-CD3 monoclonal antibodies were found to be immunosuppressive, and tolerance induction by anti-CD3 antibody treatment was demonstrated for solid organ transplants in several experimental rodent systems. In addition, antigen analogs have been found to antagonize T cell signals and specifically inhibit T cell activation. These observations have generated considerable interest in the therapeutic potential of TCR antagonists as blocking or tolerizing agents. Synthetic analogs that mimic structural features of CD4 are also being studied to block CD4 function during initial T cell activation. In principle, such limited interference with normal signals would induce tolerance of the alloreactive T cells during a short window of tolerance susceptibility, without impairing the ability of other CD4 T cells to respond subsequently to antigenic challenge. This result has been obtained in many animal model systems.

Regulating T Cell Responses Through Costimulatory (Signal 2) or Adhesion Molecules

A number of *in vitro* studies have demonstrated that prevention of Signal 2 during stimulation of Signal 1 can result in long-term T cell tolerance. A number of candidate costimulatory molecules that might be targeted for tolerance induction have been proposed; some are soluble, such as IL-1 and IL-12, and many are T cell surface receptors, such as CD28, LFA-1, CD2, HSA, CD44, and CD40 ligand (CD40L). Each has the ability to augment the T cell proliferative response to antigenic stimuli. However, the mechanism by which each acts is probably distinct, including molecules that deliver costimulatory biochemical signals to the T cell, those that enhance adhesion to antigen-presenting cells, and those that mediate homing to target tissues.

The most promising T cell costimulatory target for therapeutic intervention is the CD28 surface molecule, which interacts with B7-1 or B7-2 proteins on antigen-presenting cells. It has been shown that blocking CD28:B7 interaction during TCR engagement results in a state of antigen-specific tolerance. Similar results were also obtained recently with anti-CD40L reagents used as transplant tolerogens. Importantly, prevention of CD40L:CD40 interactions during kidney transplantation in monkeys resulted in greatly prolonged allograft survival without the need for immunosuppressive drugs. These examples suggest that tolerance induced by transient blockade of costimulation during initial antigen recognition may well be a feasible method to induce transplant acceptance without lifelong immunosuppression in humans.

One difficulty arising from the participation of multiple receptor-ligand pairs in T cell responses is that it is not clear which of the various costimulatory receptors must be blocked under clinically relevant conditions to achieve optimal endpoints. Thus, it is important that efforts be devoted to rigorous analysis of combined therapies in small and large animals, both in the presence and the absence of conventional immunosuppression. Ultimately, evaluation of these agents needs to be carried out in patients who are not undergoing therapy with pan-T cell-reactive drugs such as OKT3, cyclosporin A, or FK506, because these drugs inhibit TCR Signal 1, which is required for tolerance induction in the absence of Signal 2.

Regulation of Cytokine Networks

In addition to Signals 1 and 2, soluble mediators such as cytokines, chemokines, soluble receptors, and prostaglandins can act locally within the graft to modulate the immune response. Measurements of these soluble mediators might provide a sensitive assay for rejection *versus* tolerance and provide evidence concerning mechanisms. Although analysis of peripheral blood has not yet yielded sensitive and specific indices of rejection, analysis of intragraft cytokine production has provided some insights. For example, in some though not all studies, IL-2 and IFNγ expression was associated with rejection, while the expression of IL-4 and IL-10 correlated with graft acceptance, suggesting that the Th1/Th2 paradigm of T cell subsets (Chapter 4) might apply to transplant tolerance. Although this interpretation may be too simplistic, such results support further efforts to identify patterns of soluble mediator expression that correspond to rejection *versus* acceptance of different graft types. If reproducible expression patterns can be identified, new methods might be developed to diagnose the onset of rejection, to predict long-term tolerance, or to understand the mechanisms of rejection. Furthermore, the central regulatory role of cytokines in T cell responses makes them natural targets for gene therapy to provide local immunosuppression within a graft.

Donor Cells as Mediators of Tolerance

In animal models of allogeneic bone marrow transplantation, T cell-depleted donor marrow was shown to induce specific tolerance to donor antigens, thus permitting acceptance of highly immunogenic tissues such as skin and organ grafts from the same donor. Donor marrow-derived cells persist for long periods in such recipients, a state known as chimerism. In humans, emigration of donor leukocytes from organ grafts can also lead to a lasting state of chimerism. It is speculated that chimerism results in donor-specific tolerance, although it is also possible that chimerism simply reflects adequate immunosuppressive therapy. In human organ graft recipients, attempts to correlate chimerism with *in vitro* donor-specific hyporesponsiveness have not yielded clear results.

Bone Marrow and Cord Blood Transplants

Several approaches have been developed to increase the donor pool for allogeneic bone marrow transplantation. A registry of potential donors has been established, with greater than 3 million participants, and more than 5,000 bone marrow transplants have been carried out using matched unrelated donors identified through the registry. In the mid-1980s, umbilical cord blood became a feasible alternative source of hematopoietic stem cells. Since then, more than 200 cord blood transplants have been performed between related individuals, and over the past 4 years, more than 6,500 units of placental blood have been banked and greater than 350 unrelated cord blood transplants have been performed worldwide. These transplants have demonstrated that cord blood contains sufficient stem cells to reconstitute children and that complete HLA matching is not essential for a successful transplant. Cord blood may offer significant advantages over bone marrow, and as genetic engineering technology advances through the next decade, basic questions about hematopoiesis will be answered in the clinic by genetically marking transplanted

stem and progenitor cells, and corrective gene therapy will be undertaken using stem cell transplants either *in utero* or in early infancy.

GVHD remains a barrier to allogeneic bone marrow and cord blood transplantation, although new drug regimens have brought significant improvement in prophylaxis. Advances in basic immunology during the past decade have increased our understanding of the pathophysiology of GVHD, particularly the importance of cytokines during acute GVHD. The inflammatory cytokines TNF and IL-1 are now recognized as critical effector molecules whose blockade can attenuate the morbidity and mortality of acute GVHD in animal models. There are important relationships among the intensity of conditioning regimens, the number and function of T cells responding to host antigens, and other signals that trigger the final inflammatory cascade. Clinical studies are currently focusing on the neutralization of inflammatory cytokines by administering monoclonal antibodies, genetically engineered soluble receptors, or naturally occurring receptor antagonists for the prevention or treatment of GVHD. Another new direction is the *in vitro* alteration of donor T cell cytokine secretion prior to transfer to prevent GVHD. Increased leukemia relapse rates after T cell-depleted allogeneic transplants have illustrated the clinical value of non-T cell-depleted allogeneic marrow transplantation as an immunotherapy and the need to understand, direct, and optimize this graft-*versus*-leukemia activity as well as to progress in developing effective preparative regimens.

Xenografts

Elucidation of the molecular basis for hyperacute xenograft rejection has allowed the development of specific measures to prevent this early response. For example, synthetic, immobilized Gal-alpha-1-3-Gal has been used to remove the antibodies that initiate rejection. In another approach, pigs were made transgenic for an enzyme that inhibits production of the Gal-alpha-1-3-Gal epitope. The incompatibility of pig complement-regulatory proteins with the human complement system was addressed by genetically engineering pigs to express human complement-regulatory proteins and thus inhibit complement-mediated damage. As a result of these and other advances, hyperacute rejection is no longer viewed as the major hurdle to successful xenotransplantation, and acute vascular rejection (AVR), which resembles vascular rejection of allografts, appears as the next barrier to clinical application. AVR is associated with diffuse intravascular thrombosis thought to be secondary to endothelial cell activation. AVR may be caused by residual xenoreactive antibodies and activation of small amounts of complement. Recent studies have shown that depletion of xenoreactive antibodies or inhibition of their synthesis together with control of complement may prevent the development of AVR.

If both hyperacute rejection and AVR are avoided, a xenograft is still presumably subject to cellular rejection. It is not yet known whether the cellular rejection of xenografts is similar to T cell-mediated rejection of allografts and whether the immunosuppressive drugs used in allotransplantation would prove effective in xenotransplantation. Among the differences of potential importance are

(1) antigen presentation by xeno-MHC *versus* allo-MHC, (2) the ability of xenogeneic costimulatory molecules to react with human ligands, (3) the spectrum of additional foreign antigens in xenogeneic tissues, (4) the relative importance of induced antibody responses *versus* T cell responses, and (5) functional noncomplementarity of receptor-ligand pairs that regulate immune responses.

In addition to the immunological issues summarized above, there are at least two other important considerations for the clinical application of xenotransplantation. First, there is the possibility of physiological incompatibility, such as the incompatibility of complement-regulatory proteins. For example, pig thrombomodulin was recently found to interact poorly with human thrombin and protein C, leading to defective activation of protein C and thus to a procoagulant state. Whether an animal organ will ever fully replace the function of a human organ is still uncertain. However, it is possible that such limitations might be addressed through genetic engineering of the animal donor or through specific pharmacological therapies.

Another major limitation at present is the possible transmission of infectious disease from the xenograft to the recipient. Certainly, the potential risk to the recipient can be weighed against the potential benefit of the transplant for the individual. Of greater concern, however, is the possibility that a novel infectious agent might be transferred from the graft recipient to the human population at large. Because the recipient is likely to be immunosuppressed, the danger of animal-to-human and subsequent human-to-human infection is increased. Furthermore, transfer of retroviruses from pig to human might escape detection for years, thus complicating monitoring and raising epidemiological concerns. The extent of the risk to the public is now a subject of intensive investigation.

Summary

Of the challenges currently facing transplantation scientists, one of the most compelling is how to take the rapidly expanding information from experimental models to clinical application. Emphasis must be placed on extensive utilization of patient material from many clinical trials to determine efficacy of drug therapy, progression and immunological basis of rejection, and new approaches for centralized support of these activities. Important contributions in this area will include the development of good surrogate markers of tolerance, such as novel approaches to detect and evaluate the status of antigen-specific lymphocytes, DNA chip technology to assess immune patterns rapidly, and further development of animal models. In addition, we must develop prospective studies in humans to examine tolerance induction in the post-therapy setting and compare protocols for tolerance induction. This must all be done in the context of continuing basic research to define the underlying mechanisms of T cell activation, tolerance, and graft rejection. Other compelling challenges include the rational design of new therapeutic agents and overcoming the hurdles to the use of animal organs and tissues for clinical transplantation.

Research Opportunities

Mechanisms of Allotransplant Rejection

- Chronic rejection: characterize mechanisms and develop preventive therapies
- Characterize the roles of defined T cell subsets (CD8, CD4, Th1, Th2, etc.) in the mediation of acute GVHD, chronic GVHD, and graft-*versus*-leukemia responses

Induction and Maintenance of Transplant Tolerance

- Develop genetically engineered antibodies and small organic molecules that target early T cell signal transduction pathways or that mimic TCR antagonists to identify more effective immunosuppressive or tolerogenic agents
- Define the individual roles of CD28, CTLA-4, and CD40L in regulating tolerance induction in small and large animal models; identify new receptor-ligand interactions that promote or inhibit T cell tolerance
- Further investigate inhibitors of T cell Signal 1 or Signal 2 as tolerogens during antigen stimulation of T cells
- Investigate the expression and function of cytokines in transplant rejection and tolerance and how the balance of inflammatory *versus* suppressive cytokines may determine transplant outcomes
- Identify tolerogenic cells in bone marrow
- Establish reliable assays for the identification of tolerant states that can be applied clinically
- Develop methods to induce B cell tolerance to prevent acute vascular rejection

Improvement of Transplantation Therapies

- Apply *ex vivo* expansion technology to cord blood and marrow cells to increase available donor cell numbers and accelerate engraftment after transplantation
- Demonstrate the feasibility of gene therapy to prolong graft survival, and identify candidate molecules for gene therapy in transplantation
- Develop appropriate protocols for the transplantation of additional cells and tissues, such as muscle, cartilage, and neural tissue
- Develop or improve large animal models to test new therapeutic agents in allo- and xenotransplantation in the presence or absence of currently used immunosuppressive drugs

Xenotransplantation

- Address the physiological and infectious disease hurdles to xenotransplantation
- Define the mechanisms of T cell rejection of xenografts *versus* allografts; develop approaches to inhibit T cell xenogeneic responses such that xenotransplants can be used as an alternative to allotransplants

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Tumor Immunology

10

Overview

Immunology and cancer research, two extraordinarily complex fields, have cross-fertilized each other since the times of Ehrlich. On one hand, for example, studies in cancer research played an important role in the discovery of the major histocompatibility complex, the lymphocyte subsets, natural killer cells, and important cytokines and chemokines. Furthermore, serum from animals or humans with multiple myeloma provided, for the first time, large quantities of homogeneous antibodies for use in structural studies. On the other hand, immunological tools were instrumental in the discovery of important cancer genes such as p53, and specific T cell probes have helped identify important novel mutations that would be difficult to find by nonimmunological molecular techniques. Monoclonal antibodies have provided important diagnostic and therapeutic reagents for cancers, and recent studies suggest that major advances in the diagnosis and treatment of micrometastatic disease, a key obstacle to the cure of cancer patients, are possible. Continued cross-fertilization between the two highly developed fields of immunology and cancer research is likely to be very productive.

While T cells and antibodies enable us to resist illness from myriad infectious agents, only a minority of cancers, those that are caused by viruses, are effectively prevented by T cell-mediated immunity, although it is unclear how many cancers never reach a detectable stage because of other host defenses. When the biology of cancer and the regulation of the immune system are better understood, immune activation may lead to more effective prevention or therapy of cancer. Cancers generally harbor, and are caused by, multiple cancer-specific mutations of genes, some of which may encode tumor-specific antigens. These antigens might stimulate immune responses and serve as targets for the destruction of cancer cells. In addition, antigens that are not tumor-specific, since they are expressed on at least a subset of normal cells (tumor-associated antigens), may also serve as useful targets for therapy, if the antigens on the normal cells are sequestered, expressed at lower levels, or not presented on the surface.

There are considerable opportunities and challenges ahead. The opportunities derive from advanced understanding of the immunological mechanisms that either enable the immune system to recognize and destroy antigenic tissues or prevent this from happening. This new knowledge is being translated into numerous novel approaches to the immunotherapy of cancer. Further opportunities derive from the large number of potential target antigens on tumors and novel approaches to vaccination. The challenges derive from the need to evaluate the feasibility and efficacy of new procedures. Since clinical studies require many years, it is essential that, before clinical trials begin, appropriate preclinical studies in animal models be used

and/or developed that utilize primary or slow-growing, long-term established transplanted tumors that more closely resemble tumors found in patients than do rapidly growing transplanted tumors. Further challenges come from the need to understand better the tumor-promoting pro-angiogenic and tumor-inhibiting anti-angiogenic effects of inflammation in cancer, the mechanisms of immunological tumor destruction, and the diverse mechanisms by which cancer cells escape immune attack.

Identifying the genetic origins of tumor antigens may lead to the discovery of genes important in oncogenesis and of new targets for immunotherapy. The immune system appears to be effective against certain virus-associated cancers but ineffective against the vast majority of human cancers, which do not have an obvious viral etiology. Nonviral malignancies, however, have antigens that could be useful targets for effective immunoprevention or therapy. Certain tumors, such as bladder cancer, colon carcinoma, human melanoma, renal carcinoma, and B cell lymphoma, have responded clinically to immune-based therapies. However, much more information is needed on the nature of antigens present on common human cancers and on the mechanisms that allow or prevent immunological tumor rejection. Despite the significance of CD4 T cells in promoting the intensity and duration of cytotoxic CD8 T cell responses, relatively little attention has been paid to the identification and role of CD4 T cell recognition of tumor antigens. Many new immunotherapeutic approaches have been proposed, far too many to be tested clinically in an effective way. Therefore, *in vitro* systems and animal models are needed to test which antigens and immunological approaches can be used during the many steps of cancer development to achieve (1) prevention of cancer development, (2) eradication of dormant cancer cells, (3) eradication of residual cancers after surgery, and (4) rejection of late established cancers (Figure 10-1).

Tumor Antigens

Table 10-1 lists examples of known tumor antigens. Antigens encoded by viral genes will be discussed below under *Immunoprevention*. Nonviral tumor antigens can be divided into two major groups: those that are "tumor-specific" and those that are not; the latter are called "tumor-associated." A number of tumor-associated antigens have been identified, and several appear to be recognized by autologous T cells and/or antibodies. All tumor-associated antigens are expressed on at least some adult nonmalignant cells. Some of these antigens are found on normal precursor cells of the tissue of tumor origin and thus represent differentiation antigens. Other tumor-associated antigens are not tumor cell lineage-specific but, in terms of nonmalignant adult cells, have only been found in cells of the testes and placenta that do not express MHC antigens. Tumor antigens expressed on the cancer cells but not on normal cells can be used to identify which patients have micrometastatic disease following cancer surgery and need further treatment to prevent recurrence or relapse of the cancer. Experimental and clinical studies have suggested that expression of an antigen on normal adult cells is not necessarily an impediment to its use as a therapeutic target against cancer, especially if its tumor expression

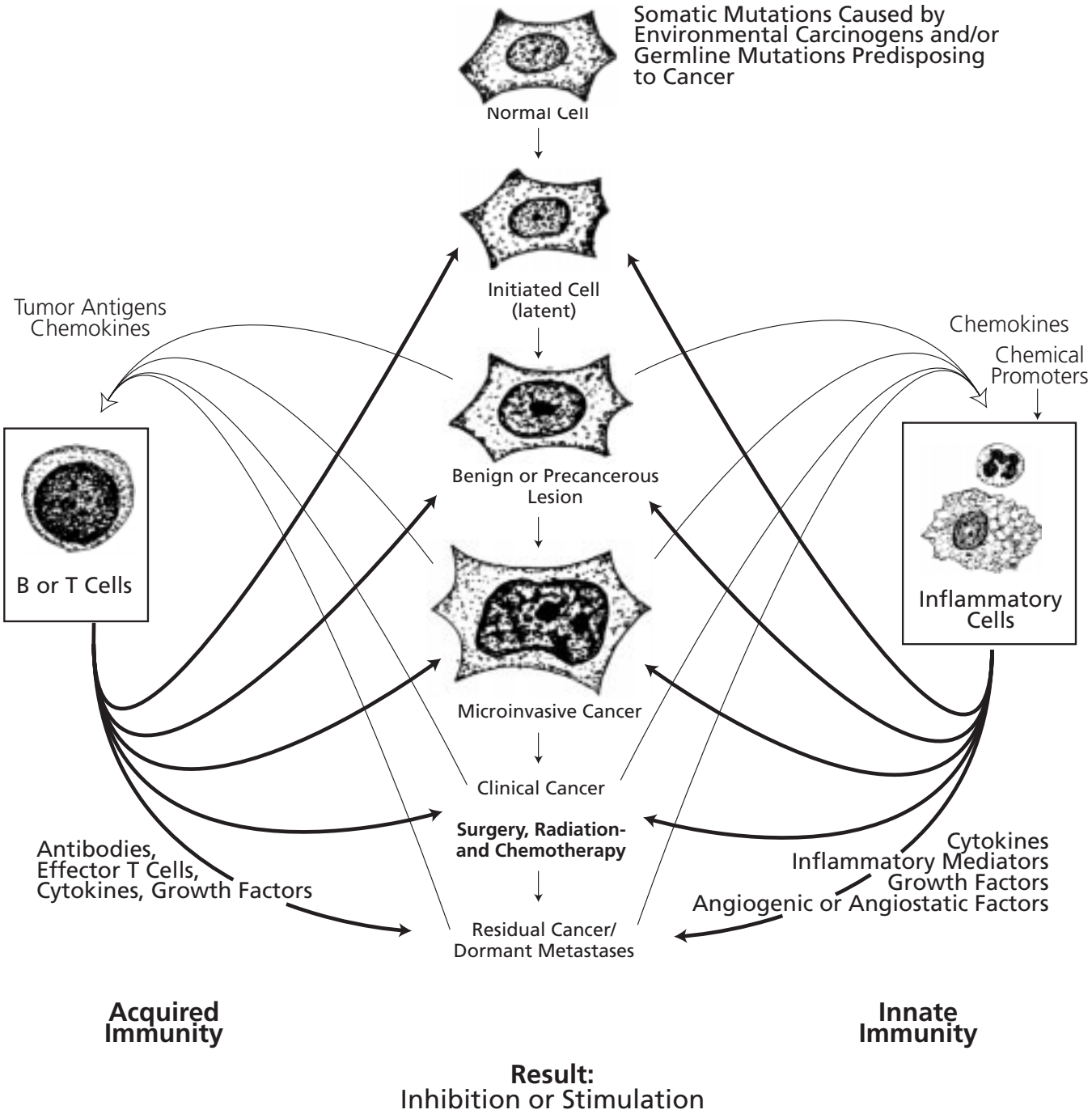

Figure 10-1. Cancer development, cancer progression, and the immune system. Acquired and innate immunity can have either stimulatory or inhibitory effects. (From Schreiber, H. Tumor immunology. In: *Fundamental Immunology*, 4th ed., Paul, W. (ed). New York: Raven Press, 1998, in press; reprinted with permission.)

Table 10-1. Orgin, Distribution, and Antigenicity of Different Categories of Tumor Antigens Represented by Examples.

Category	Type of antigen	Example	Mechanism of expression in cancer	Contributes to malignant behavior	Normal adult tissue distribution	Occurrence				Recognition by		Rejection antigen
						Cancer-specific	Normal precursor lineage	Type of cancer	Shared versus unique	T[a]	B	
I. Normal cellular gene products												
	Oncosper-matogonal antigen	MAGE-1	Ectopic expression	?	Testis	—	—	Several	Shared	+		
	Oncofetal antigen	MAGE-3	Ectopic expression	?	Testis/ trophoblast	—	—	Several	Shared	+	—	
		P1A	Ectopic expression	?	Testis/ trophoblast	—	—	Mastocytoma	Shared	+	—	
		CEA	Amplification of regenerative cell	?	Colon/ lactating breast	—	+	Several	Shared	—	+	Weak
	Differen-tiation antigen	17-1A	Normal surface glycoprotein	—	Broad	—	+	Several	Shared	—	+	Passive
		PSA	Normal intra-cellular enzyme	—	Prostate	—	+	Prostate	Shared	—	—	
		Tyrosinase	Normal intra-cellular enzyme	—	Melanocytes	—	+	Melanoma	Shared	+	+	Passive
		Lewis (carbohydrate)	Aberrant glycosylation	?	Broad	—	+	Several	Shared	—	+	
		HER-2/neu (oncoprotein)	Overexpression	+(?)	Breast/ ovary	—	+	Several	Shared	—	+	
		GD2/GD3 ganglioside	Overexpression	?	Broad	—	+	Several	Shared	—	+	Passive
	Clonal antigen	Immuno-globulin idiotype	Clonal amplification	—	B cell clone	—	+	B cell malignancy	Unique	(+)	+	Weak
II. Mutant cellular gene products												
Primary detection as antigen:												
	Ribosomal protein	Mut. L9	Point mutation	LOH	—	+	—	Fibrosarcoma	Unique	+	+	
	Cyclin	Mut. cdk4	Point mutation	+	—	+	—	Melanoma	Highly restricted	+	?	
Primary detection as oncogene:												
	Oncogene product	Mut. p21s	Point mutation	+	—	+[b]	—	Several	Shared	+	+	?
	Suppressor gene product	Mut. p53s	Point mutation	+	—	+[c]	—	Several	Unique	?	+	?
	Internal fusion protein	Mut. EGFR	Internal deletion	+(?)	—	+	—	Ganglioma	Shared	?	+	?
	Chimeric fusion protein	BCR-ABL	Translocation	+	—	+	—	CML	Shared	+	+	?
III. Viral gene product												
	Nuclear protein	E6/E7 of HPV16	Transforming viral gene	+	—	+[b]	—	Cervical	Shared	+	+	+

[a] Abbreviations are: Mut., mutant; T, T cells; B, B cells; MCA, methylcholanthrene; CML, chronic myelogenous leukemia; EGFR, epidermal growth factor receptor; HER-2, human epidermal growth factor receptor 2; LOH, loss of heterozygosity; PSA, prostate-specific antigen; HPV, human papillomavirus.

[b] Can also occur in carcinogen-induced benign premalignant lesions (e.g., papillomas).

[c] Also found as germline mutations in the dominantly inherited Li-Fraumeni familiar cancer syndrome.

From Schreiber, H., Tumor immunology. In: *Fundamental Immunology*, 4th ed., Paul, W. (ed). New York: Raven Press, 1998, in press. Reprinted with permission.

exceeds that of normal cells by two or three logs, and provided that some destruction of the normal tissue would not cause undue toxicity. However, peripheral tolerance that inactivates high-affinity T cells may be a significant problem, and evidence for the efficacy of tumor-associated antigens as therapeutic targets is incomplete.

Most human cancers in developed countries are thought to be induced by physical or chemical carcinogens. Experiments in mice have shown convincingly that the rejection antigens recognized by the host's immune system on tumors induced by certain carcinogens are antigenically distinct between individual tumors. It has become clear that these often highly immunogenic, so-called "unique" antigens are truly tumor-specific, since they appear to be encoded by somatic tumor-specific mutations that are absent in any autologous normal cells. Several such tumor-specific antigens have now also been discovered on human malignant cells. Finally, several of the mutant genes may play a critical role in the establishment of malignancy and its progression. These unique antigens might not be selected against during tumor progression if they are essential for the maintenance of malignant growth.

Animal Models

In general, it can be said that many different treatments are effective in causing tumor rejection in animal models when given before or early after tumor challenges. However, most treatments are ineffective against established macroscopic tumors. While some experiments suggest that this problem is not primarily related to the growing number of tumor cells, most models do use rapidly growing tumor cells and long-term, persistent cancer cannot be studied. In contrast, most human tumors probably grow much more slowly than serially transplanted animal tumors, and human tumors may be dormant or persist as residual cancer after therapy for many months to years (Figure 10-l). Therefore, new animal models that permit study of the therapy of dormant cancer cells or minimal residual disease are needed. It is quite possible that the requirements to eradicate these cancers by immunological means are different from those needed to eliminate rapidly growing cancers. Many studies suggest that the stroma plays a major role in the establishment of cancers, and the local environments of recent *versus* long-established tumors may be distinctly different. In experimental models, metastatic cells can be prevented from growing by anti-angiogenic factors resulting from the presence of the primary tumor, and such micrometastases can be susceptible to immunological destruction following active immunization as long as it occurs immediately after the primary tumor is removed. In humans, passive treatment with antibodies to certain tumor antigens might be effective in eradicating early micrometastatic spread if treatment is given soon after removal of the bulk of the tumor. There is a clear need for the development of animal models of dormant micrometastatic disease.

Mechanisms of Immune Destruction

The desired end point of immunotherapy is tumor regression and rejection. In animal models, CD8 T cells are known to be essential for immunological rejection of

many ultraviolet induced and chemically or virally induced cancers. However, the mechanisms by which these T cells are effective *in vivo* are not understood. It is now known that the CD4 T cell subset can also be critical for antitumor immunity, particularly for the development of CD8 T cell memory and for survival of adoptively transferred CD8 T cells. NK cells are effective in experimental models in eliminating circulating cancer cells and can recognize cancer cells that have lost expression of one or more MHC class I molecules (see below). Tumor antigen-specific antibodies can also lead to the destruction of micrometastatic cancer cells *in vivo*.

While antigen-specific responses may result in more permanent antitumor effects, such responses are likely to be ineffective if not induced in the proper inflammatory environment, or if they fail to induce the effector mechanisms of innate resistance, such as macrophage activation and the production of anti-angiogenesis factors or cytokines with antitumor activities. Immunotherapeutic approaches, such as treatment with the mycobacterium bacille Calmette-Guérin (BCG), may induce their antitumor effects *via* nonspecific mechanisms. For example, repeated instillation of BCG into the bladder has become the treatment of choice for superficial bladder cancer after surgery. The infection leads to a prolonged inflammatory response in the bladder wall and reduces the incidence of recurrence. It has been known since the clinical studies of Coley over a century ago that inflammation, as induced by the intratumoral application of bacterial vaccines under certain conditions, can have antitumor effects *in vivo*. Various inflammatory cells, including neutrophils and macrophages, can be activated with bacterial substances to kill cancer cells *in vitro*. Furthermore, several cytokine gene-transfected tumors induce inflammatory reactions and show decreased tumorigenicity. Experimental evidence suggests that NK cells, macrophages, and various proinflammatory cytokines, such as INFγ, IL-12, and TNF, can be critically involved in the rejection of tumor cells *in vivo*, for example, by inducing anti-angiogenic factors such as IP-10. The challenge is to decipher the relative importance of various components in the complex interactions among multiple cell types and humoral mediators that occur in the tumor stroma during rejection.

Escape of Tumors From Immune Destruction

Tumor development is best characterized as a stepwise evolution of clonal subpopulations that grow more effectively in the host (Figure 10-1). Growth advantages can result, for example, from total or selective loss of MHC class I antigens, from β_2-microglobulin mutations, and/or from antigen-processing defects, all of which occur more frequently in metastatic tumors. These abnormalities can prevent the recognition of malignant cells by cytolytic T cells because of inefficient antigen presentation. Defective antigen presentation may be particularly important as an escape mechanism for tumor cells that cannot lose expression of antigens that are essential for maintaining the malignant phenotype. Lack of costimulatory molecules on cancer cells may lead to peripheral anergy of T cells, and expression of Fas ligand by tumor cells may lead to apoptosis of the T cells entering the tumor. Tumor-infiltrating inflam-

matory cells such as macrophages, which locally release certain cytokines such as TGFβ, IL-10, pl5E, or PGE_2, may contribute further by downregulating immune cell function. It is also known that large tumor burdens can lead to systemic defects in TCR or FcγR signaling pathways in both mice and humans. Furthermore, a sufficient number of highly antigenic tumor cells might vigorously stimulate reactive T cells to die after activation, leading to immune exhaustion and outgrowth of the tumor. Interestingly, complete surgical excision of a growing tumor from experimental animals is often sufficient to provide long-term, specific immunity against rechallenge, suggesting that clonal exhaustion might be a temporary, reversible occurrence. The advanced age of many tumor-bearing patients may be an additional important factor in reducing the effectiveness of immune responses to tumor antigens, because immune competence declines with aging.

Thus, it has become clear that multiple mechanisms can explain the loss of antitumor immune function at the site of tumor growth or systemically in later stages of cancer. Since clonal evolution and mechanisms of tumor progression may be different for the same tumor type in different patients, the mechanism(s) by which a tumor escapes immune recognition might be quite different among patients carrying the same tumor type. Therefore, individualized therapeutic approaches and simultaneous use of multiple target antigens might decrease the likelihood of escape from immune recognition. It is important to state that the immune system itself may hamper effective immunotherapy and immunoprevention. Clearly, the presence of certain T cell subsets can interfere with adoptive cellular immunotherapy, and the presence of certain CD8 and/or CD4 T cell subsets may correlate with tumor escape. Furthermore, antibodies elicited by a tumor may positively or negatively regulate T cell responses to the tumor. Elucidation of the mechanisms responsible for the downregulation of effective immune responses in cancer patients is critical for the rational design of immunological therapies.

Tumor Promotion and Tumor Progression

It has become increasingly clear that inflammatory responses can either enhance or inhibit tumor development and growth. Initial evidence suggests that the chemokine, cytokine, and growth factor environments may differ in tumor-promoting and tumor-inhibitory conditions of inflammation. Tumor-promoting effects have been suggested by numerous studies (Figure 10-1). The mechanisms involved are being studied using transgenic and gene knockout mice as well as other methods. Transgenic cancer-prone mice can be shown consistently to develop skin tumors after wounding or application of a chemical promoter. In another model, inhibition of prostaglandin synthase was found to prevent the development of intestinal neoplasms. Interestingly, in addition to directly affecting cell growth and blocking IL-12 and IFNγ production, prostaglandins can induce angiogenesis, and these studies suggested that interstitial cells in intestinal polyps are major producers of prostaglandin E_2. Among the interstitial inflammatory cells, neutrophils and macrophages can also produce cytokines and factors that favor vascularization. In addition, MCP-1,

which blocks the production of proinflammatory cytokines such as IL-12, IFNγ, and TNF, may be present. While the above suggests the importance of a certain type of inflammatory response in "tumor promotion," which is the development of a neoplasm from latent, initiated cells, recent work suggests that the second major stage of tumor development, called "tumor progression," can also be aided by inflammation. For example, the progression of cancer from a less aggressive to a more aggressive stage can be associated with the development of variant cells that have a greater ability to attract inflammatory cells and to be growth-stimulated by products of these inflammatory cells. Finally, inflammatory cells are important in enabling cancer cells to metastasize and are therefore important in the late phases of tumor progression. Clearly, we need to increase our understanding of the inflammatory mediators and regulatory circuits involved in controlling tumor growth. Because of the magnitude of the effects of inflammation on cancer development, learning to manipulate inflammation may help develop powerful new approaches to prevent and treat malignant diseases.

Immunoprevention

There is convincing evidence that immune surveillance prevents the development of tumors expressing the major Epstein-Barr virus (EBV)-encoded antigens targeted by cytolytic T cells. Thus, EBV-associated B cell lymphomas that present the full array of EBV-encoded target antigens appear only in immunosuppressed patients. There is also good evidence that adoptive T cell immunotherapy can be effective, even when these cancers are well established. It is not clear whether other virus-associated human cancers can also be treated effectively by adoptive T cell immunotherapy at late stages. Furthermore, it is not known whether human papillomavirus (HPV)-associated cervical cancer can be prevented by active immunization of patients at early stages of the disease, before carcinoma *in situ* or invasive cancer occurs. Certainly, prevention of infection with the tumor-inducing virus by prior immunization would be the most effective approach, and immunization is being tested for HPV-associated diseases and for hepatitis B and C virus-associated hepatocellular carcinoma. Other cancers, such as Kaposi sarcoma and human T cell leukemia associated with HTLV-1, offer additional opportunities for the development of immunoprevention therapy. Further work is needed to determine whether cancer in individuals carrying a predisposing mutation might be prevented by immunization; premalignant cells in these individuals may express tumor-specific antigens encoded by the mutant genes.

Immunotherapy

Multiple immunotherapeutic approaches have been developed and some of these may evolve as effective ways to control cancer. At present, however, very few of these therapies appear to be highly effective or are the treatment of choice. One example to be discussed below is the treatment of micrometastatic disease in patients with resected colon cancer. Another example, discussed above, is the successful use of BCG for treating patients with residual superficial bladder cancer. As an alternative approach, researchers have transfected various genes into tumor cells that are then lethally irra-

diated and used as vaccines. Several transfected cytokine and costimulatory molecules have been reported to increase tumor immunogenicity. GM-CSF appears most promising, because this cytokine promotes the recruitment, maturation, and activation of dendritic cells (DC), which are powerful antigen-presenting cells that could pick up shed tumor antigens and stimulate tumor-reactive T cells. It has already been shown in animal models that DC pulsed *in vitro* with virus-specific or tumor-associated peptides will induce tumor-reactive T cells and cure animals bearing transplanted tumors. DC have also been loaded with peptides eluted from the MHC molecules of tumor cells and used as specific, customized therapeutic vaccines. In this case, the peptides need not be identified; the pool of eluted peptides can clearly bind MHC and is assumed to include relevant targets for tumor immunity. In another approach, DC loaded with heat shock proteins complexed to tumor-specific peptides showed enhanced antigen presentation and tumor rejection. Finally, DC have been successfully transfected with tumor-derived RNA to present tumor-specific peptides. The advantage of these approaches appears to be the potential for inducing powerful immunity to either tumor-specific or tumor-associated antigens without the need for direct antigen identification. A limitation of these approaches is that the immunogen cannot be standardized. Other novel approaches include tumor DNA vaccines and the use of anti-idiotypic monoclonal antibodies that bear the internal image of tumor-associated antigens. Such antibodies were shown to induce immune responses to nominal antigens in colon carcinoma and melanoma models.

Many of the above approaches are highly therapeutic in experimental tumor models when used within the first few days or weeks after tumor transplantation. However, it remains to be proven that any of these vaccination procedures will be clinically effective on long-established cancers. It should be understood that in addition to prophylactic vaccination, tumor immunologists are attempting active therapeutic vaccination, a procedure that has been largely abandoned in the clinical management of infectious diseases. Tumor cells, like certain infectious agents, may escape surveillance by inducing a deviant, ineffective immune response or by inducing active suppression or tolerance. Cancer cells have a slower generation time than most infectious organisms, and often the bulk of tumor load can be removed by therapy. At the time when the antigen load is at the lowest level, the suppressive environment might be modifiable, and active immunization could lead to an effective therapeutic immune response. The critical question, therefore, is whether residual or dormant cancers can be treated effectively with active immunization.

Because of these uncertainties with active immunization, some researchers have placed more emphasis on the development of passive antibody therapy or adoptive transfer of T cells stimulated *in vitro*. A monoclonal antibody directed against a tumor-associated antigen on colon cancer cells has been effective in reducing the incidence of micrometastatic spread when treatment is begun shortly after surgery at early stages of tumor growth (e.g., in patients with resected Duke C colorectal cancer). A number of B lymphoma patients treated with an anti-B

cell monoclonal antibody have had significant remissions, and the antibody is now FDA-approved and in clinical use for non-Hodgkin's B cell lymphoma. In addition, idiotype-specific antibodies against B cell lymphomas are being tested as tumor-specific therapies. Antibodies are also being used as vehicles to carry a cytokine or toxic agent to kill tumor cells, or are being made bispecific to bind both tumor antigen and effector killer cells. An alternative approach is the use of recombinant antibody:cytokine fusion proteins to concentrate cytokines in the tumor microenvironment. Humanization of mouse monoclonal antibodies has increased the efficiency of these approaches by limiting anti-antibody responses. The clinical efficacy of these diverse antibody-based therapeutic reagents cannot be properly evaluated until phase II/III trials are conducted.

Adoptive therapy with T cells stimulated *in vitro* has not yet become a standard therapeutic approach for cancer but appears to be highly effective against human EBV-associated malignancies. One of the major problems is the lack of information on which antigen will be the most effective target molecule and at which stage of the disease therapy should be initiated. Antigenic heterogeneity is frequently encountered with human solid tumors as a consequence of their inherent genomic instability and this poses a major obstacle to therapy aimed at a single antigen. Thus, targeting multiple independent antigens seems preferable. Animal experiments have unequivocally demonstrated the efficacy of targeting either viral or unique tumor-specific proteins as rejection antigens but also suggest a role for targeting shared, nonmutated tumor-associated antigens in immunotherapy. Therapeutic approaches utilizing commonly expressed tumor antigens lend themselves to generic, and hence more convenient, strategies. However, individualized customized approaches for human cancer immunotherapy that would target unique antigens are being developed. Certainly, therapeutic efficacy, and not convenience, cost, or marketability, must be the primary determinant for selecting the optimal therapeutic approaches to be developed.

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Research Opportunities

Tumor Antigens

- Determine whether tumor-specific unique antigens are commonly encoded by genetic mutations that play a functional role in the establishment and progression of malignant disease
- Determine the genetic origins of tumor antigens recognized by tumor-specific CD8 or CD4 T cells to identify novel mutations important in the development of malignancy
- Determine why tumor-associated antigens or tumor-specific mutant proteins encoded by conventional oncogenes or tumor suppressor genes have not been found as primary targets of immunological tumor rejection after immunization with cancer cells
- Determine whether amino acid sequence-based prediction of epitopes can be used to identify effective immunological targets
- Determine the parameters that predict the relative effectiveness of tumor-specific or tumor-associated antigens in eliciting tumor rejection and serving as targets for active therapeutic immunization or adoptive immunotherapy
- Determine the usefulness of certain tumor antigens (e.g., oncospermatogonal) for detecting micrometastatic disease

Animal Models

- Develop animal models of tumor dormancy, microscopic or macroscopic residual tumors, and late-established tumors in which immunotherapeutic approaches can be tested
- Determine whether primary cancers in cancer-prone animals can be prevented by immunization against tumor-specific antigens encoded by, or induced by, the predisposing mutation
- Determine whether primary, long-established, slow-growing transplanted cancers can be prevented by immunization against tumor-associated or tumor-specific antigens
- Determine whether primary or long-established, slow-growing transplanted cancers can be treated by immunotherapy alone or in combination with surgery or other treatments

Mechanisms of Tumor Destruction and Escape

- Identify the differences between the mechanisms involved in tumor-stimulatory, pro-angiogenic *versus* tumor-destructive, anti-angiogenic inflammatory responses
- Design approaches directed toward promoting the infiltration of tumor-specific T cells into tumors and their reactivation and survival at this site

- Develop ways to increase the intensity and duration of cytolytic T cell responses by stimulating tumor-specific CD4 helper cells
- Develop models to break tolerance and induce high-affinity T cells specific for tumor-associated antigens that are also expressed on normal cells
- Better define the biologic significance of potential autoimmune reaction to tumor-associated antigens; define conditions under which autoimmune responses can provide effective antitumor immunity
- Characterize the origin and maturation stages of dendritic cells that are effective in inducing antitumor responses and search for modifications that make such DC even more effective.
- Determine the influence of CD4 and CD8 T cell subsets producing different cytokines on immune responses to cancers

Preventive Immunization and Immunotherapy

- Develop effective methods for preventive and/or therapeutic vaccination against virus-associated human cancers
- Develop methods to prevent cancer by immunizing against tumor-specific mutant proteins in cancer-prone individuals
- Develop immunization approaches that allow simultaneous induction of immune responses to multiple antigens; determine whether immunodominance by one or more epitopes diminishes responses to other epitopes; and determine whether avoidance of immunodominance prevents tumor escape
- Develop immunological methods to eliminate micrometastatic disease in patients following cancer surgery
- Develop conditions that permit the adaptation of common human cancers to tissue culture to facilitate more detailed studies of potential therapies in humans
- Develop ways to fund academic phase II/III trials based on scientific rather than business considerations

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Reproductive Immunology

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Overview

Both the male and female reproductive tracts are mucosal surfaces that interface with a potentially hostile external environment. The columnar epithelium of the uterus and fallopian tubes contains intraepithelial lymphocytes that may be hormonally regulated and may include such functions as cytotoxicity, cytokine production, and maintenance of the epithelium. The genital tract mucosa is part of the "common mucosal immune system," and immune responses generated at a distant mucosal surface, such as the intestine or lung, may appear in the reproductive tract in the form of primed T and B cells, the latter usually producing IgA.

Interestingly, the gametes produced in the testis and ovary are antigenic to their own host. Furthermore, the female recipient of semen can mount an immune response to soluble antigens in seminal plasma leading to anaphylaxis, or to spermatozoa leading to antisperm antibodies and infertility. The product of conception is semiallogeneic and will also elicit immune responses. In fact, successful pregnancy in outbred populations is a prime example of immune tolerance. Despite the potential for destructive immunity, immune responses in the context of reproduction are thought to be averted, suppressed, or deviated toward a beneficial outcome by poorly understood mechanisms. A particular type of immune response to antigens in male ejaculate may actually enhance development of fetal trophoblast cells that invade the uterus to establish a placenta, and in so doing, may prevent the development of high blood pressure with kidney failure and seizures in late pregnancy. Factors in seminal plasma may inhibit Thl-type immunity in the female and enhance implantation; this may be due in part to seminal plasma TGFβ (a Th3-type cytokine), which alters APC, T, and B cell responses. TGFβ also may stimulate uterine epithelium to produce GM-CSF, which attracts and/or activates macrophages and other cells beneficial for implantation.

Monthly shedding of the uterine lining occurs in humans and other primates, and retrograde passage of uterine epithelium into the abdominal cavity can lead to an inflammatory response in some women that is typified by Thl-type cytokines leading to infertility. Endometrial cells may implant, and further inflammation may lead to scarring and adhesions (endometriosis). Related problems such as pain, infertility, and intestinal obstruction may require costly medical and surgical therapy. Of particular interest are γδ T cells that recognize heat shock proteins expressed on damaged epithelium and may play a role in the local immune response/local inflammatory response in endometriosis. Sexually transmitted, disease-causing microorganisms enter *via* reproductive tract surfaces. The nature of protective immune responses is still uncertain, and a mucosal vaccine to such pathogens as HIV would likely prove

more effective than a parenteral vaccine. Overpopulation represents a major world problem that has fueled searches for safe, effective, and reversible forms of immunocontraceptive vaccines. The only successful vaccine to date has been based on immunizing women to the hormone hCG produced by fetal trophoblasts. Protection is less than 100 percent but sufficient to reduce the frequency of pregnancies. A gamete-antigen-based vaccine is also under development and will be discussed below. Finally, recent results suggest a role for immunity in preventing teratogenesis; this finding is intriguing because it has an impact on the problem of birth defects and their prevention. It is clear that many immunological parameters are involved in successful human reproduction, and a variety of opportunities to advance this important field are now available through new technological approaches.

Gamete Immunology

It is important to note that 10 percent of U.S. couples suffer from infertility. Although some infertile couples will be effectively treated by *in vitro* fertilization and embryo transfer or by intracytoplasmic sperm injection to introduce male gamete DNA into the oocyte *in vitro*, there is still a need to define the molecular defects that cause infertility. Immune responses to gametes, such as spermatozoa, testis, ovary, oocyte, and its casing of zona pellucida, have been studied for many years as a possible cause of infertility. This research has implications for diagnosing and treating spontaneous infertility; for infertility arising from reproductive tract trauma, infection, or surgery such as vasectomy; and for the identification of antigens for use in immunocontraceptive vaccines. Immune responses to antigens of the zona pellucida have been studied in mice, and the immunogenetic basis for the response that causes oophoritis and irreversible ovarian failure has been determined. A peptide vaccine that induces reversible infertility without such complications was developed for use in humans and proved effective in clinical trials.

Ten percent of infertility is thought to be attributable to immunological causes. Clearly, immune responses to spermatozoan antigens can lead to infertility. However, there has been controversy concerning the role of spontaneously arising antisperm antibodies in the male and/or female as a cause of infertility. Antisperm IgG and IgA antibodies have been detected in the genital tract, and plausible mechanisms of action can be postulated, although the antigenic determinants have not yet been defined. However, the mere presence of antibody has been taken as evidence of pathogenicity, and the statistical issues in achieving fertilization have not been considered. Even if 90 percent of the 160 million spermatozoa in an ejaculate were coated with antibody, the 16 million unaffected gametes still would exceed the 2 to 5 million threshold required for fertility. Thus, an exclusive focus on antibodies should be avoided; other parameters of immune involvement should also be further investigated. For example, it is known that cytokines and macrophages are required for normal testicular and ovarian function in mice, as well as for implantation, and certain Th1-type cytokines interfere with gamete functions and with development of the early embryo after fertilization.

A major step forward in the area of contraception would result from determining which epitopes/antigens could be useful as targets for immunocontraceptive vaccines. Furthermore, once the specific antigen is identified, there is also potential for developing more precise diagnosis and treatment. Modifications of the sperm plasma membrane in the milieu of reproductive tract fluids are tied to fertilizing ability, and new proteins, such as CD52, are inserted during epididymal passage. Some of the carbohydrates on these CD52 molecules appear to be unique, and monoclonal antibodies to CD52 block fertilization and cause agglutination. There are also sperm antigens involved in the acrosome reaction, which is required for penetration of the zona pellucida surrounding the oocyte, and at least one antigen is acquired in the epididymis that decapacitates, that is, induces quiescence. The molecules thought to recognize oocyte receptors after passage through the zona include SP17, zona receptor kinase (p95), fertilin (PH30), and SP10. Molecules involved at the time of binding to the zona include CD46 and P56. Many of these molecules have yet to be cloned, characterized, and tested as immune targets.

Opportunities exist for both clinical application and commercial development. For example, monoclonal antibodies to sperm proteins could offer an effective immunological spermicide that avoids the problems of current spermicides, which contain a detergent (N-9) that desquamates the vagina, leading to irritation and an increased risk of transmitting sexually transmitted diseases. Although antibody preparations are expensive, detailed information on their specificities could lead to development of more economically feasible mimetic compounds that block sperm antigens. The challenge for human immunocontraception is to find the optimal mixture of antigens for maximal efficacy. Furthermore, a focus on antigens required at the earliest steps of fertilization would increase the likelihood of wide acceptance. Finally, elucidation of the mechanisms of gene regulation in the testis and epididymus offers the possibility of new leads for male contraception.

Immunogenetic Aspects of Pregnancy

Three areas have been the focus of much of the clinical and experimental work in reproductive immunogenetics: the etiology of recurrent spontaneous abortions, the control of MHC antigen expression in the placenta, and the relationship between recurrent pregnancy loss and autoimmunity. The latter issue will be addressed in a separate section. Recurrent spontaneous abortion is a heterogeneous complex of diseases whose proximate cause (pathogenesis) and ultimate cause (etiology) have yet to be clearly elucidated, but both genetic and immunologic factors can be involved. Studies in rats have identified MHC-linked genes that affect reproduction, and studies in humans have shown a relationship between sharing of the HLA B-DR-DQ region and recurrent spontaneous abortion, unexplained infertility, and decreased fecundity. Statistical analysis of the HLA data in recurrent spontaneous abortion and in unexplained infertility indicated that the genes involved most likely were not the HLA genes themselves but closely linked genes. Experimental studies also showed that lethal epistatic interaction can occur between MHC-

linked genes and genes elsewhere in the genome. Evidence for the involvement of immunological factors in some types of abortions has been provided by the prevention of abortions using appropriate immunization protocols in mating studies of certain mouse strains. For example, abortion has been prevented in DBA/2 mated CBA/J and B10.A mated B10 mice by immunization against paternal MHC antigens, and abortion mediated by poly I:C-activated natural killer (NK) cells can be blocked by immunization with allogeneic MHC-transfected fibroblasts. The control of MHC antigen expression in the placenta is complex and involves constitutive suppression of class II antigens, inducible suppression of the polymorphic class I antigens, and genomic imprinting such that only the paternal class I antigens are expressed. Much speculation has been raised about the role of placental monomorphic class I MHC antigens (called Pa in the rat and HLA-G in the human) in affecting the immune response to the trophoblast, and further study is clearly warranted.

One cogent problem is to define the relative roles of genetic defects and immunological mechanisms in recurrent spontaneous abortions and to determine whether patients can be stratified into predominantly genetic or immunologic etiologies to guide the choice of potential therapies. A genetic etiology would most likely involve mechanisms active at the time of fertilization or shortly thereafter that would have their primary effects on the process of implantation. An attractive working hypothesis is that genetic defects influence the expression and function of intercellular adhesion molecules that would disrupt the orderly assembly of cells in the embryo or perhaps elicit a destructive immune response in the mother. An immunological etiology would involve the control of trophoblast antigen expression and its temporal variation, particularly that of the monomorphic MHC class I antigens, and the consequences of different class I MHC antigen expression in terms of eliciting a destructive or protective maternal immune response. Further work is needed to define these control mechanisms. Immunotherapy for recurrent spontaneous abortion using allogeneic lymphocytes has been effective in some strains of mice and in some cases in humans, and should be explored further with an emphasis on whether the effect is immunogenetically specific or whether the liberation of particular cytokines following an immune reaction is the crucial element. Of interest, it has been shown that immunotherapy can prevent teratogenesis in mice. Improved understanding and more effective treatment of recurrent spontaneous abortions are increasingly important, because the average childbearing age of the population is increasing and reproductive problems are becoming more cogent. Subjecting a woman to an ineffective therapy or rescuing a fetus with severe genetic defects is not a desirable outcome.

Immunological Mechanisms of Premature Birth

Low birth weight is the single greatest cause of perinatal morbidity and mortality in the United States. Health problems related to premature birth can be quite severe, and the total financial cost of supporting children with this disorder is substantial. There are two major causes: (1) preterm birth due a potential variety of

immunological, hormonal, endocrinological, infectious, and nutritional causes, and (2) small-for-gestational-age infants resulting from intrauterine growth retardation, with or without preeclampsia, or congenital anomalies. Recent research has implicated immunological mechanisms, mediated through cytokines and/or antibodies, as a major contributing factor in both situations. In addition, maternal infections are frequently associated with preterm births.

The first step in the prevention of low birth weight infants is to identify women who are at risk by developing appropriate markers for this disease and by validating them in large populations of prenatal women. Candidate markers that have been reported to be potentially useful are serum alpha-fetoprotein, estradiol, ferritin, IL-1, IL-2, IL-6, TNFα, and PAPP-A (pregnancy-associated plasma protein-A). The presence of increased serum levels of alpha-fetoprotein, endothelial cell activators, soluble TNF receptors, hCG, IL-2, and IL-6 has been associated with intrauterine growth retardation and with preeclampsia. The second step in preventing preterm births is to have a reliable surveillance system for the early detection of embryos with congenital anomalies, particularly aneuploidies, since such anomalies are associated with preterm birth. A recent study has shown that screening for PAPP-A and hCG is an effective approach to detecting trisomies at 10 weeks. The role of cytokines in the maintenance of pregnancy needs to be better defined so that potential aberrations in their production can be evaluated as a cause of preterm births. Aberrations in cytokine production could be induced by genetic abnormalities in the regulation of placental antigen expression and its subsequent effects on immune responsiveness, or could be induced by infection or stress, which are frequently associated with preterm birth.

Maternal Lymphomyeloid Cells and Cytokines

In normal human reproduction, 50 percent of fertilized oocytes fail before or at the time of implantation, 15 to 20 percent fail as occult pregnancies, and 5 to 10 percent become obvious miscarriages. There is also significant loss in certain mating combinations of inbred mice and in domestic animal reproduction; for example, pigs lose 10 to 20 percent. The very high rate of loss in humans is due in large part to chromosomal abnormalities in the fertilized oocyte. In addition, however, current evidence indicates that maternal immune responses can play an important role, in some instances, in causing failed pregnancies even with normal fetal karyotypes, and that alternative responses may be essential for successful pregnancy. The immunobiology/physiology is different in the preimplantation period, the implantation and early periimplantation periods, the placental phase, and the late placental phase when fetal growth is the main event.

Lymphomyeloid cells in the uterus were initially of interest as indicators of an immune response in the endometrium, and more recently, as potentially important participants in fetomaternal immunoregulation. In the human endometrium, lymphomyeloid populations of importance include $CD56^+$ $CD16^-$ NK cells. There are also a small number of T cells, predominantly $\alpha\beta$ and

γδ CD8 cells, as well as $CD16^+$ NK cells, macrophages, and mast cells. Intraepithelial lymphocytes represent the first site of contact with the embryo, and cytotoxic activity is downregulated at the time of implantation. The laboratory rodent has been widely used in the study of pregnancy immunology and it differs from the human. The rodent equivalent of the human $CD56^+16^-$ cell population needs to be clarified. In both the human and mouse, the uterine NK-lineage, αβTCR and γδTCR populations expand during pregnancy. In the mouse, the NK cells develop into large granulated cells that congregate on the mesometrial side of the uterus where they have been viewed as a "gland." In the human, where there is a hemochorial villous placenta and extravillous trophoblast invades diffusely into the uterine decidua, granulated cells do not show such enlargement and are found in all areas. The TgE26 mutant mouse lacks NK cells in the uterus and has a small placenta, and abortions can be corrected by grafting NK cells. The NK cells may facilitate trophoblast growth by secreting vasodilatory nitric oxide and cytokines.

Human $CD56^+16^-$ cells may produce a variety of cytokines, including CSF-1, GM-CSF, IFNα, IFNγ, LIF, and TGFβ1, that play a role in pregnancy. Secretion of GM-CSF and a TGFβ2-like immunosuppressive factor by human $CD56^+16^-$ cells or mouse γδ, NK and γδNK cells may be of particular importance to successful pregnancy, as shown by studies in pregnant mice. Cytokines may also be produced by a variety of nonlymphomyeloid cells in the uterine lining. The epithelium is important for TGβ2, CSF-1, and LIF production; endometrial stromal cells can produce IL-8; and IGF-type molecules may also be produced and affect trophoblast growth. Both lymphomyeloid and nonlymphomyeloid cells may be under hormonal control. Progesterone receptors on activated CD8 T cells in murine and human pregnancy provide a mechanism whereby gestational hormones can enhance secretion of a factor that suppresses NK cell activity. Pregnancy deviates immunity toward Th2/3 responses both systemically and in the uterus, and may downregulate CD4 and CD8 expression on T cells specific for paternal alloantigens. The mechanisms of these effects are unknown. Systemic changes during pregnancy may be beneficial, as exemplified by remission of rheumatoid arthritis in humans and collagen-induced arthritis in mice, or may be harmful, as reflected in a reduced ability to cope with infections that require a Thl-type response.

The vasculature in the decidua is very important to pregnancy outcome. In mice, embryo demise is caused by TNFα, IFNγ, and IL-1 activation of vascular endothelial prothrombinase, which causes clotting and necrosis. How the expression of prothrombinase is regulated merits investigation. Stress is a well-known abortogen in animal systems and is suspect in human recurrent abortions and prematurity/low birth weight. Recent experimental data in mice indicate that substance P released from uterine neurons acts on macrophages, mast cells, and CD8 T cells to bias the uterine environment toward a Thl cytokine pattern.

Chronic Uterine Infections

It is now well established that viruses, bacteria, and mycobacteria can be present in the chorioamnion and amniotic fluid as early as 12 weeks of human gestation even when membranes are intact. These organisms are accompanied by a low-grade inflammatory response and can persist for up to 34 weeks' gestation. The immunological consequences of intrauterine infections have not been studied, and it is not known whether such infections result in induction of tolerance to unrelated antigens or in hypersensitivity or aberrant antibody responses. It is known that the functions of macrophages, polymorphonuclear leukocytes, NK cells, and T cells are downregulated in the uterus, and trophoblast-derived IFNγ and IFNα have been proposed to defend against infection. Intrauterine infections often lead to chronic pelvic inflammatory disease which, in turn, leads to a high incidence of maternal morbidity and sterility.

Placental Cells and Cytokines

The placenta is a complex organ, and there are anatomical differences among species. In sheep, the trophoblast produces IFNτ, which maintains ovarian hormone production needed for pregnancy. In the mouse, no INFτ is produced, and the pituitary sustains the ovary until placental trophoblast can produce its own hormones. In humans, the villous trophoblast produces hCG to stimulate ovarian hormone production. The migratory properties of extravillous human trophoblast cells appear to depend on the types of surface integrin molecules that are expressed, and such expression can be affected by cytokines such as TGFβ1 and TGFβ3. Human extravillous trophoblast invades decidua and the walls of spiral arteries; a similar arterial invasion occurs in mice.

Placental cytokines may act in an autocrine, paracrine, and perhaps endocrine manner. For example, IL-6 may enhance hPL secretion by human placental trophoblast; CSF-1 and GM-CSF may promote trophoblast differentiation and peptide hormone secretion; IL-1, TNFα, IL-8, and IFNγ may act on cells in adjacent decidua; and GM-CSF may stimulate bone marrow/spleen growth in pregnant mice. Insulin-like growth factors may be produced by, and act on, both trophoblast and decidual cells. Upregulation of IL-8 secretion by trophoblast might provide a trigger for premature parturition, and endometrial IL-8 may cause abortions.

The placental trophoblast serves as a selective barrier between the maternal and fetal circulation. Trophoblast cells transport certain types of maternal IgG into fetal tissue. Maternal lymphomyeloid cells also cross, but there may be a selection against primed T cells by trophoblast-associated Fas ligand. The fetus has mechanisms for suppressing and/or eliminating certain maternal cells that pass beyond the placenta, but it is clear in mice that some types of maternal cells can persist in the fetal bone marrow. The nature and effect of such cells on the developing immune system are uncertain. Maternal cell entry into the fetus is thought to explain fetal tolerance of the noninherited maternal MHC haplotype in outbred matings. Fetal cells also cross the trophoblast into the mother, and it has been proposed that passage of MHC class II allogeneic fetal cells into the mother

may enhance the remission of diseases such as rheumatoid arthritis during pregnancy.

Autoimmune Diseases and Pregnancy

Epidemiological studies have indicated an increase in the prevalence of recurrent pregnancy losses in women with autoimmune diseases. Pregnancy has also been associated with the transient remission of autoimmune disease. Very little is known about the mechanisms of these pregnancy effects. Clinical studies in humans and experimental studies in animals suggest that MHC genes play an important role. One particularly interesting association is supported by recent work indicating a causal effect of HLA-DR and -DQ disparity between mother and fetus and the remission of symptoms in rheumatoid arthritis. The mechanism is not yet known. It might be explained if fetal allopeptide binding to maternal HLA molecules competes with maternal peptides to reduce the activation of maternal T cells by self-peptide-HLA complexes. Such a mechanism would provide strong impetus to investigate the possibility of exogenous peptide treatment to ameliorate autoimmune disease. It is also possible, however, that the association might be due to other genes in the MHC, and not to HLA antigen presentation. Alternatively, pregnancy might provide a cytokine environment that inhibits the cohort of normally pathogenic autoreactive T cells and stimulates a separate population of cells that secrete more benign effector molecules to reduce disease.

One basic problem in exploring such MHC-linked associations is to define more clearly the etiology of the specific autoimmune disease and to differentiate it from its pathogenesis. Many diseases that have an immunological component in their clinical manifestations may not have a primarily immunological etiology. A classical example is tuberculous pneumonia in which the tubercle bacillus is the primary cause of the disease, and the resulting immune response is the pathogenesis of tissue destruction and the clinical symptoms. Clarification of these issues would greatly aid in defining the specific consequences of pregnancy on underlying disease.

The investigation of autoimmune diseases associated with recurrent pregnancy loss is a fertile area in which to pursue future studies, because much is known about both processes in humans and because there are excellent animal models in which to investigate the association. In humans, a thorough genetic epidemiological study investigating the incidence of recurrent pregnancy losses in women with autoimmune diseases such as rheumatoid arthritis, diabetes, and autoimmune thyroiditis and the distribution of these diseases in their families and in the families of their spouses is necessary to provide further clinical and basic information upon which to develop etiological and pathogenetic studies at the cellular and molecular levels. The investigation of the reproductive capacity in rodents that develop autoimmune diseases would provide the first step in the identification and analysis of these disorders from both the immunological and genetic perspectives.

Transplacental Immunization

Clinical studies with tetanus toxoid in humans and experimental studies with a variety of antigens in animals have demonstrated that immunization of the mother during pregnancy can actively sensitize the fetus, as demonstrated by IgM antibody formation in the fetus and by a memory response in the offspring up to 1 year after birth. The effects of transplacental antigen stimulation can last up to 10 years in humans, as shown by the persistence of increased lymphocyte responsiveness to tetanus toxoid; tolerance was not induced. However, antibody responsiveness was not affected. Both the human and animal studies showed that the critical criterion for effective transplacental immunization was that the antigen must be given in an insoluble form as a precipitate, absorbed onto alum or in an oil adjuvant. Metabolic studies in rats showed that this form of immunization was essential to provide a steady level of persistent antigen stimulation to avoid the induction of tolerance by antigen overload, and that the transplacentally passed antigen became localized to the reticuloendothelial system of the liver, spleen, and bone marrow. Carbohydrate antigens generally induced tolerance in the fetus unless given as a protein conjugate.

Neonatal deaths from a variety of infectious diseases, and the transplacental transmission of HIV leading to illness and death at an early age, are major medical and public health problems worldwide. Transplacental immunization offers the possibility of preventing intrauterine infection and of enhancing immune responsiveness in the neonatal period due to active immunization of the fetus.

Because of some past untoward experiences with vaccines using attenuated or killed infectious agents, only inert substances should be used in vaccines employed for transplacental immunization. A variety of inert immunogens are currently available: (1) proteins: tetanus, diphtheria, pertussis, cytomegalovirus, hepatitis B, and experimentally, malaria, cholera, and HIV; and (2) carbohydrates (which are best used as protein conjugates): group B streptococcus, meningococcus, gonococcus, *H. influenzae*, and pneumococcus. Recent advances in identifying antigenic epitopes that elicit the best protective effects against specific organisms, the use of genetic engineering and new adjuvants, and the use of DNA vaccines will greatly expand the potential scope of transplacental immunization. Clearly, much research remains to be performed before effective transplacental vaccines become commonplace.

HIV Infection

HIV infection now occurs commonly on a heterosexual basis, with the male as the donor. It appears that the bacterial status of the female reproductive tract can affect susceptibility. Nonhuman primate studies suggest that alloantibody in the female might reduce the chance of infection. HIV-positive mothers may pass the virus to their offspring either *via* transplacental passage during pregnancy or *via* breast feeding. Anti-HIV drug therapy can reduce the rate of infection from mother to infant to about 10 percent; potentially, immunotherapies such as transplacental immunization might further reduce this rate.

Animal Models

Studies in animals have already allowed us to dissect many of the immunological and genetic events involved in normal pregnancy and in recurrent pregnancy loss. For example, mouse models of the immune response to the fetal placental unit and the manipulation of this response to prevent some kinds of spontaneous abortions have provided paradigms for subsequent studies in humans. Studies of the MHC-linked *Grc* genes in the rat and the *T/t*-complex in the mouse have provided substantial insights into reproductive genetics and are serving as prototypes for clinical studies.

The critical approach is to select the correct animal model to study a particular reproductive problem. Rodents are most often used, since their hemochorial placentas are similar to those of humans, they are immunologically well characterized, and they can be genetically manipulated. Farm animals can only be used in more restricted settings, but their use has contributed greatly to knowledge in reproductive immunology and genetics. Nonhuman primates are ideal for a number of studies, particularly those involving vaccination against infectious diseases, but due to expense they can be used only in very clearly defined and closely monitored settings. Comparative studies in several species have often provided crucial insights.

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Research Opportunities

Fertility

- Characterize the mucosal immune response in the male and female using animal and human studies to elucidate beneficial responses during insemination and pregnancy-related immune tolerance
- Determine the specific roles of cytokines in regulating mucosal immune responses, and develop potential therapeutic approaches to modulate cytokine activity for optimal reproductive health
- Characterize the types of immune responses required for immune protection against infections of the reproductive tract, including chronic infections
- Clarify the role of immune factors in the prevention of teratogenesis
- Identify the antigens and immune responses that prevent hypertension in pregnancy
- Study the role of intercellular adhesion molecules such as the integrins and adhesins in implantation and early embryogenesis
- Clarify the functions of MHC-linked genes that affect fetal development

Contraception, Infertility, and Spontaneous Abortion

- Develop specific immunodiagnostic reagents for rapid evaluation of male infertility
- Develop immunologically based topical spermicides and inexpensive mimetic compounds that block sperm antigens; characterize appropriate sperm antigens for development of immunocontraceptive vaccines
- Determine the relative roles of genetic factors and immunological mechanisms in recurrent spontaneous abortion in both humans and animal models
- Identify the molecular mechanisms of spontaneous abortion and the roles played by HLA-G and the polymorphic MHC antigens in promoting successful pregnancy
- Develop novel immunotherapeutic approaches for the prevention of spontaneous abortion
- Characterize the molecular processes by which stress contributes to infertility and unsuccessful pregnancy

Premature Birth

- Develop reliable diagnostic markers for preterm birth risk and rapid screening methods for clinical use
- Determine the molecular basis for immune induction of preterm births; investigate the roles of specific cytokines and the cells that produce them to identify targets for immunomodulation

Mechanisms of Maternofetal Tolerance

- Further identify, quantitate, and characterize the functional importance of cytokines and cytokine combinations active at the fetomaternal interface
- Define the roles of T cells, NK cells, and macrophages in promoting successful outcomes during each phase of pregnancy

Pregnancy and Disease

- Identify the cellular and molecular mechanisms responsible for pregnancy-related remission of autoimmune disease
- Determine the genetic loci and molecular mechanisms responsible for the association between recurrent spontaneous abortion and autoimmune diseases
- Investigate basic mechanisms for the induction of immunity *in utero*; characterize methods of antigen transport and presentation and the functional capacities of fetal lymphocytes
- Develop more effective methods for antigen delivery and adjuvant formulation to optimize transplacental immunization; conduct clinical trials of transplacental immunization with currently available antigens
- Develop animal models for the study of immune involvement in eclampsia/preeclampsia, intrauterine growth retardation, and premature delivery
- Characterize the functional consequences of microchimerism resulting from the engraftment of fetal cells in the mother
- Investigate the effects of external influences on the developing embryo and fetus using animal models, with a focus on the pathogenesis of infectious diseases such as HIV, malaria, and hepatitis and the development of vaccines against such diseases

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Neuroimmunology

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Overview

Work in the past decade has provided intriguing new information on signaling between the nervous system and the immune system. We now know that cells of the immune system express receptors for numerous neurotransmitters and neurohormones and that they respond to at least some of these agents *via* classical signal transduction pathways. Because much of this information was obtained by exogenous treatment of experimental animals with the relevant substances, their precise effects under physiological conditions are important topics for future investigations. The most prominently investigated neural-immune signaling routes are those associated with the "stress" axes: the hypothalamo-pituitary-adrenal (HPA) axis and the hypothalamo-sympathetic axis. A variety of stressors are known to induce profound alterations in specific immune responses and in the outcome of disease, and the mediators responsible for these alterations are beginning to be defined.

We also know that immune-associated cytokines, especially IL-1, IL-6, and TNFα, can act on central nervous system (CNS) circuitry and can elicit a variety of behaviors, including components of "illness behavior," activation of the HPA axis, and activation of sympathetic outflow, especially to lymphoid organs. These neural effects can be evoked by very small concentrations of cytokines. Recent work suggests that inflammatory cytokines and other immune-derived molecules may contribute to the pathology of neurodegeneration in Alzheimer's disease. Past studies have shown that cells of the immune system can synthesize and release molecules that are neurotransmitters or neurohormones and that neurons and glia can synthesize and release cytokines, thus suggesting a commonality of signals shared by these two systems. Research activity in this field is growing rapidly, utilizing both *in vitro* and *in vivo* animal and human model systems.

Nervous System Effects on the Immune System

Neural Receptors on Cells of the Immune System

It is known that cells of the immune system express receptors for many neurotransmitters and neurohormones. Some of these receptors are expressed only at specific times during development, or during specific stages of activation, and may be restricted to naive or memory lymphocytes, or cells at a specific anatomical location. Descriptive work is still needed to identify more fully the sequences, structures, and expression parameters of these receptors on immunocytes, and the signal transduction pathways induced by receptor engagement need to be defined in greater detail. Of particular interest is the interplay of signals generated by neural receptors with those induced by T cell receptors, B cell receptors, cytokine receptors, or colony-stimulating receptors. It is clear that neurotransmitters and neurohormones

influence the more conventional immune signal transduction pathways, but the nature, extent, and dose-response characteristics are poorly understood at present. Such knowledge may allow therapeutic modulation *via* synergistic effects of neural signaling together with colony-stimulating factors or cytokines for bone marrow stimulation, for activation of specific effector T cells in cancer chemotherapy, or for the deliberate skewing of cytokine production toward Th1- or Th2-type dominance in autoimmunity.

Neural-type Ligands

The ligands that interact with neurotransmitter receptors on immune cells have been found in nerve fibers that distribute into the parenchyma and along the smooth muscle compartments of lymphoid organs. Past work focused on sympathetic noradrenergic nerve fibers and some peptidergic fibers such as those releasing substance P (SP) or vasoactive intestinal peptide (VIP). We now need a more extensive knowledge of which ligands are available in which compartments at particular periods of time or activation to better understand when ligand-receptor signaling actually occurs *in vivo*. Studies are needed to identify the ligands present, their neuronal cells of origin, and the connective wiring back into the central nervous system to understand the circumstances that lead to their functional activation. Pharmacological studies to determine the range of interactions possible for a given ligand on specific subsets of lymphoid cells are also needed. There have been surprisingly few dose-response studies of this nature, and even fewer that relate the findings to physiological systems of activation.

We know that some immune cells can synthesize and release specific neurotransmitters and neurohormones, especially following stimulation by a virus or specific activation signal. For example, beta-endorphin and adrenocorticotropic hormone are produced by leukocytes after stimulation with corticotrophin releasing factor (CRF), which is produced by macrophages or nerve cells following IL-2 stimulation. It appears that a variety of neural-type ligands are available for interactions with immune cells, and that some of these ligands are produced by immunocytes themselves. We have only scratched the surface in identifying these signals and relating them to specific immune responses and their roles in human disease.

Neural Signaling, Immune Responses, and Disease

Neurally derived compounds, such as norepinephrine or SP, can act on specific subsets of lymphoid cells to alter their activity. Many of these neurally derived signals are known to increase during specific behaviors, during stress, or during central nervous system (CNS) outflow to peripheral structures. More information is needed on the actual concentrations and duration of these mediators in the blood and specific lymphoid organs during ongoing physiological activity, or ongoing disease processes such as viral or bacterial infection, remission or exacerbation of autoimmune disease, chronic inflammation, or during cancer chemotherapy. Interestingly, it has been shown that a stressor interferes with the ability of cytoxan to eliminate a weakly immunogenic pulmonary tumor. Although little is yet known, neurotransmitters found in nerves, such as SP, norepinephrine, and

neuropeptide Y, and in primary lymphoid organs, such as oxytocin, vasopressin, and CRF, may influence the proliferation and differentiation of maturing T cells in the thymus or of lymphocyte, granulocyte, or other hematopoietic lineages in the bone marrow.

Both catecholamines (from sympathetic nerves) and glucocorticoids (from the HPA axis) can exert influences on T helper cell production of cytokines. We need to explore more carefully the physiological presence and function of these neural mediators in specific disease states, and their role in shifting cytokine production toward Th1- or Th2-dominant responses. Glucocorticoids, catecholamines, and other neuromediators can modulate the severity of autoimmune diseases. Autoimmune-susceptible animals, such as Lewis/N rats, are deficient in CRF and activation of the HPA axis. Moreover, nonsusceptible strains, such as Fisher 344 rats, become autoimmune-susceptible when the HPA axis is blocked. In addition, it was found that blocking of sympathetic nerves or catecholamines in the lymph nodes leads to an earlier onset and more severe course of autoimmune arthritis, whereas blocking of SP fibers protects against autoimmunity.

Glucocorticoids and catecholamines also appear to play a role in immune senescence. In old rodents, sympathetic nerves are lost selectively in the spleen and lymph nodes. Furthermore, experimental removal of these nerves in young rodents leads to markedly diminished cell-mediated immune responses, such as those needed to clear pulmonary viral infections, thus mimicking changes seen with natural age-related nerve loss. Restoration of these sympathetic nerves with growth factor stimulating agents restores IL-2 production and cell-mediated immunity.

Natural killer (NK) cells are highly manipulable by catecholamines and glucocorticoids, which affect both NK cell number and activity. Results in animal studies indicate that NK cell activation can protect against tumor growth, and in humans, breast cancer and many metastatic processes are known to be NK cell sensitive. Interestingly, NK cell activity in women with breast cancer appears to vary inversely with past stress. Because of the prominent role of catecholamines and glucocorticoids in stress responses, relaxation responses, exercise, laughter, and a variety of environmental and behavioral responses, a better understanding of their role in altering immune responses for protective benefit is needed. Such studies will require analysis of the molecular mechanisms that underlie the action of the neural mediators.

Neuroendocrine Effects on Immunity

The hormone, cortisol, and its analogues are known to influence immune responses, and high doses are used clinically for immunosuppression in autoimmune disease and transplant rejection. However, cortisol has some immune-enhancing effects at physiological concentrations, and in stressor paradigms it mediates only some of the immunosuppressive effects of the stressor. More information is needed on the interactions of glucocorticoids with cells of the immune system at physiological concentrations. Much is already known about the effects of opioid peptides on a variety of molecular, cellular, and physiological parameters of

immune reactivity. However, we know very little about the physiological availability and function of opioid peptides in disease processes. Contrary to expected results, morphine administration was found to slow the progression of simian AIDS, perhaps by acting through sympathetic neural influences in lymph nodes, although other explanations are possible.

The role of estrogen and other reproductive hormones in immune responses is not well understood, and these hormones may be important in autoimmune diseases that predominate in females. The interactions of reproductive steroids with growth factors, such as the interaction of estrogen with nerve growth factor, might be relevant for protective therapy in Alzheimer's disease. It is known that growth hormone and prolactin can influence the development and aging of some specific aspects of immune reactivity, such as T cell responses and thymocyte differentiation, but the mechanisms are not yet defined. Many aspects of immune response, including lymphocyte proliferation, cytokine production, and effectiveness of steroid-mediated immunosuppression, are driven by circadian rhythms. Only some of these circadian effects can be explained by glucocorticoid rhythms, and recent attention has focused on melatonin as one diurnally driven hormone that might be involved in these circadian effects. We need to understand better the underlying mechanisms and therapeutic benefits that circadian regulation imparts to immune reactivity.

Behavioral Effects on Immunity

Stressors

Clinical studies investigating the effects of stressors such as caregiving, change in living quarters to a nursing home, divorce or marital strife, and even examination stress have often shown a correlation with diminished parameters of immune reactivity, with some indications for increased disease susceptibility. For example, prior stress can lead to increased cold virus infections. Further study is needed to clarify the mechanisms by which social support and the individual's perception of control can buffer these effects, and to identify effective interventions to prevent changes in immunity in those who are at risk during highly stressful events in their lives.

For the past two decades, much attention has focused on stressors and impaired immunity, creating the impression in both the lay and scientific communities that behavioral stressors are always immunosuppressive. The literature does not entirely support this view, and suggests that the type of stressor, its intensity, timing, duration, and context can all profoundly affect the type of immune modulation that results. Some of these responses are suppressive, but some are enhancing and others are unaltered. Too little attention has been paid to the neurally derived mediators that accompany stressors; in particular, the roles of norepinephrine, glucocorticoids, and neuropeptides are not well defined. Renewed emphasis on integrative approaches to medicine in the area of immune-related diseases should include systematic, quantitative studies of the underlying neural mediators in both animal and human models before and after pharmacological or behavioral manipulation, and careful assessment of mediator relationship to the stressor under study. This approach can be applied in cancer therapy, chronic

inflammatory states, Alzheimer's disease, atherosclerosis, autoimmune diseases, and infectious diseases. The functions of stressors and stress mediators should also be studied in chronic infectious diseases, such as tuberculosis and AIDS, and in conjunction with the effects of surgery, anesthesia, trauma, and wound healing. Interestingly, wound healing appears to be especially susceptible to the effects of stress.

Integrative Interventions

Limited studies have focused on the benefits of integrated interventions that include counseling, peer support groups, and lifestyle-altering activities, and the results suggest that such approaches can lead to enhanced quality of life and increased longevity in patients with breast cancer and malignant melanoma, although actual effects on the immune system have not been demonstrated as yet. There are additional provocative experimental results that suggest neural-immune interactions may be more broadly applicable to other types of cancers, although the particular roles of stress mediators and immune cells are not yet known. The potential effectiveness of such integrative interventions is considerable, and third-party payers are strongly recommending these approaches because of cost considerations. This area should receive further research attention from both the mechanistic and the physiological outcomes perspectives.

Immune System Effects on the Nervous System

Recent work has demonstrated that cytokines produced by cells of the immune system can exert profound effects on activity of the nervous system and on behavior. It was also found that immune-type cytokines can be produced by glia and neurons. The inflammatory cytokine IL-1, as well as other mediators produced during viral and bacterial infections, can induce a collection of responses or behaviors, together called "illness behavior," that includes reduced activity and exploratory behavior, reduced feeding, lethargy, sleep, fever, and activation of the HPA and hypothalamo-sympathetic axes. Little is known about the relevant physiological levels or the kinetics of cytokine production in human diseases. Transport carriers have been demonstrated for IL-1α and β and for the IL-1 receptor antagonist, but the concentrations of IL-1 that can be achieved in the CNS at sites where these illness behaviors are initiated are not yet known. Careful dose-response studies of IL-1 administered over time as well as in a bolus are needed to document the onset, duration, and sequence of appearance of these behaviors. There are suggestions from past work that small molecule mediators, such as prostaglandins and nitric oxide, might mediate some of the cytokine effects on neural function, and some studies have suggested that ascending noradrenergic pathways to the hypothalamus mediate cytokine effects of illness behavior and activation of the stress axes. However, contradictions occur in the literature, and some studies indicate that central noradrenergic release is a consequence of, not a cause of, cytokine actions in the brain.

Several routes of action for cytokine effects have been suggested, including direct transport across the blood-brain barrier, leakage through the circumventricular organs, binding of the cytokine to

Research Opportunities

Neural-Immune Interactions

- Assess the functional importance of molecules that mediate interactions between the immune and nervous systems under physiological conditions
- Identify functionally relevant receptors for neurotransmitters and neurohormones on cells of the immune system and for cytokines on cells of the nervous system
- Determine the intracellular signaling pathways utilized by neural-type receptors on cells of the immune system and the molecular basis for their ability to modulate other immunoregulatory signals
- Localize neural-type ligands that function in immune responses and identify their cells of origin and mode of production
- Determine which neural-type mediators act on particular lymphocyte or antigen-presenting cell subsets and quantitate mediator concentrations and duration during different types of immune responses
- Investigate the potential role of neurotransmitters in the selection and differentiation of bone marrow stem cells and thymocytes

Applications of Neuroimmunology to Human Disease

- Identify the molecular mechanisms of stress-related effects on immune function
- Investigate the role of specific immune responses within the CNS, and the role of inflammation, in modified neural functions at the molecular level
- Elucidate the mechanisms by which neuromediators influence the duration and severity of autoimmune disease
- Define the roles of catecholamines and glucocorticoids in immune senescence

organum vasculosum of the lamina terminalis with subsequent activation of prostaglandin E_2, action on mediators released from endothelial cells in the brain vasculature, activation of afferent nerves to the brain, and action through small molecules that stimulate the production and release of cytokines from central microglia and astrocytes. The intraperitoneal injection of cytokines appears to activate vagal afferents to the brain stem, whereas intravenous injection provokes different responses by different mechanisms. More comprehensive studies to compare equipotent dose administration by intraperitoneal, intravenous, and intracerebroventricular routes are needed to better understand how cytokines act in the brain.

Recent success in delaying the progression of some Alzheimer's disease symptoms with anti-inflammatory agents illustrates the potential importance of understanding cytokine-brain interactions. Cytokines and local immune responses may be important in other chronic inflammatory brain conditions, brain injury, radiation exposure, and brain cell

transplantation. Tumors and other conditions that disrupt the blood-brain barrier may expose the brain to higher than usual concentrations of cytokines from the general circulation. The role of cytokines in growth factor-induced sprouting in the central and peripheral nervous systems, and during neuroprotective and neurorestorative strategies, should be investigated.

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Immune Response to Infectious Agents

13

Overview

Despite the enormous success of numerous vaccines that are now commonly used to prevent infectious diseases, the human population remains susceptible to a broad spectrum of contagious pathogens for which no vaccines or effective treatments are available. Furthermore, variant microorganisms that are resistant to current treatments are emerging in the human population, as are microorganisms not previously known to infect humans. Given the ease of transmission of contagious microbes in an age of rapid air travel, these global changes in susceptibility to infection demonstrate the great need for continued and vigorous emphasis on new vaccine development. Clearly, HIV/AIDS serves as a stark and readily appreciated example of the great need to accelerate research and translate basic findings for the effective prevention of infectious diseases.

Continued success in vaccine development will depend not only on a greater understanding of immunological responses in different age groups, but also on expanded knowledge of immunogenic pathogen epitopes, the natural history of infection by individual microorganisms, the large number of mechanisms by which pathogens evade the immune system, and the specific types of immune responses required to eliminate pathogenicity. The widely diverse biology and biochemistry of human pathogens pose a tremendous challenge for research in the coming years, but the enormous savings in human suffering and prevention of economic losses realized from even one successful new vaccine provide strong justification for expanded investments in this area. This chapter will consider research opportunities for the prevention and cure of different types of infectious diseases. Vaccine development is covered in Chapter 14, and given our focus on the immune system, the extremely important area of antibiotic drug development is not included.

Microbial Latency and the Role of Infection in Chronic Inflammatory Diseases and Cancer

Viruses are well known for their latent persistence in the host due to their unique ability to incorporate their genetic material into host genomes. However, examples of latency can also be found in bacterial, fungal, and parasitic infections. In these cases, the mechanisms include microbe-induced immunosuppression or antigenic variation that results in evasion of the immune system. The genetic background of the host may be crucial in determining the frequency of latency for a given pathogen. The importance of microbial latency cannot be overemphasized. Latent infections can serve as reservoirs for transmission to other susceptible individuals, and they can reactivate within the original host to cause acute or chronic progressive disorders.

Latent infection might also play a role in the origins of some human cancers. According to the World Health Organization's 1996 Report, a growing number of bacterial, viral, and parasitic pathogens are significantly associated with cancer (also see Chapter 10). Many of the implicated microbes are commonly transmissible and could be treated with existing antibiotics, or could potentially be treated with antiviral reagents or prevented by vaccination. In addition, there is a growing body of evidence indicating that *Chlamydia pneumoniae* plays a role in cardiovascular disease. Proof of causality and greater understanding of pathogenesis in any one of these diseases would have great implications for public health in terms of treatment and prevention. Few areas of research hold greater promise of contributing to our understanding of infectious and chronic diseases and the eventual relief of human suffering.

Infection and Immunity

Immune cells continuously circulate throughout the body searching for invading organisms: viruses, bacteria, fungi, and parasites such as worms. Although antibodies produced by B cells are critical effector molecules for immune protection, the task of focusing an immune response appropriate to the specific type of invader resides with T cells. Antigen-specific T cells interact with B cells and other cells in the body, determine whether foreign protein antigens are present on the cells, and then organize the specific attack to eliminate the invader. In responding to infectious pathogens, T cells have two jobs that are carried out by distinct subsets. Helper T cells orchestrate the response of other immune elements, such as macrophages and B cells. Killer or cytotoxic T cells identify cells harboring invading organisms and either kill the infected cell before the invader can replicate or modify its metabolism to minimize replication of the invader. The responding T cells also divide to increase their numbers. Importantly, some of the dividing T cells become memory T cells that can live for many decades. Memory T cells are then present in sufficient numbers to eradicate invading organisms more rapidly upon reinfection. A major goal of preventive vaccination against many pathogens is to induce such memory T cells, thus allowing enhanced antibody, cytokine, and cytotoxic responses for protection. Vaccination should also induce memory B cells and long-lived antibody-secreting plasma cells.

In addition to their native forms, foreign antigens are displayed on antigen-presenting cells (APC) as small peptide fragments of the original protein bound to cellular MHC class I and class II molecules specialized for the precise purpose of presenting these peptides to receptors on T cells. Class I molecules are produced by most cell types in the body; they present peptides derived from organisms actively infecting the cells and are recognized by killer T cells. Class II molecules are normally produced only by cells involved in immune responses, such as B cells, macrophages, and dendritic cells; they display peptides derived from organisms sampled from the surrounding fluids and are recognized by helper T cells, which then give specific instructions to generate an appropriate immune response. The means by which cells generate peptides and deliver the peptides to class I and II molecules is called antigen

processing, and quite distinct mechanisms are used in class I and class II antigen processing. Increased knowledge of these mechanisms is needed to rationally design vaccines that can induce the proper T cell responses. The problem is complicated by the fact that different individuals produce different variants of class I and class II molecules, some of which bind different peptides. Although much has been learned in recent years, continued basic research is needed in this area, as are effective approaches to translate basic knowledge into clinically relevant protective and therapeutic vaccines.

Microbial Pathogenesis and the Immune System

A description of the multitude of pathogens that continue to plague the human population and their methods of infection and pathogenesis is beyond the scope of this chapter, as is a discussion of vaccine development. Rather, we provide some thoughts on general areas important for future research on different classes of infectious agents.

Bacteria

Under the rubric of "bacteria" are also included mycobacteria, rickettsiae, ehrlichiae, chlamydiae, spirochetes, and mycoplasmas. The complete DNA sequences of all important bacteria that are pathogenic for humans will be available by the end of the first decade of the new millennium. Newly developed technology will be employed to define precisely which genes are essential in the pathogenesis of infection. In particular, high-density nucleic acid arrays will be used to examine the total panoply of gene expression at different times during infection and at different tissue sites within the host. In parallel, genetic approaches will be used that can directly identify genes that are required during the infectious process; these genetic approaches complement current molecular approaches because they more directly permit the identification of regulatory pathways that are operative at specific times after infection.

By using sensitive reporter molecules, it will soon be possible to follow individual microorganisms within infected animals and establish the precise elements of the host cellular and antibody responses that are at play. Thus, the future promises to give us a portrait of the infectious process that has been heretofore hidden from view inside the animal or human host. It will become possible to focus on the earliest interactions between host and pathogen, to devise strategies to enhance innate immunity, to formulate new classes of anti-infective molecules, and to create better vaccine candidates to abort the infectious process. As we come to appreciate the nuances of host/pathogen interactions, we will obtain important information about the biology of immune defenses against invading microbes as well as about the strategies microorganisms employ to thwart our immune barriers.

Viruses

The study of viral infections has provided important information on antiviral immune responses and on some of the methods used by pathogens to evade immune destruction. A new twist was added to our understanding of viral pathogenesis with many recent discoveries of viral gene products that interfere

with host defense mechanisms. Highly selective modulation of individual antiviral immune mechanisms appears to be a common strategy of the viral genome, especially among the DNA viruses such as adenovirus, herpesviruses, and pox viruses. Some viral products have been shown to interfere with antigen presentation by MHC molecules, thus preventing T cell activation. In addition, viral inhibitors have been identified for many host immune effector mechanisms, ranging from interferons and chemokines to cytokines and cytolytic T cells. Some of these inhibitors are homologous to mammalian proteins, such as the pox virus molecules that are homologous to mammalian receptors for IL-1 and TNF and can bind and inactivate these cytokines. Other inhibitors bear no homology to known host proteins, such as the intracellular proteins of adenoviruses that prevent lysis of infected cells by TNF or Fas ligand. Viral proteins such as pox virus CrmA and baculovirus p35 that block apoptosis have already proved invaluable in delineating the steps leading to cell death induced by a variety of stimuli. It is anticipated that many more viral inhibitors of intracellular signaling pathways are yet to be discovered and that this category of proteins will provide important tools for the study of these pathways. It can also be anticipated that such viral gene products will contribute to a variety of approaches for preventing human disease, from the development of new viral vaccines, in which genes that interfere with vaccine effectiveness will be identified and deleted from the immunizing strain, to the use of viruses as vectors for gene transfer.

Fungi

The rise in number and types of immunocompromised patients, including AIDS patients, has resulted in the emergence of fungal diseases that were previously rare or nonexistent. Invasive fungi are now the fourth leading cause of nosocomial disease. Over the past few years, unprecedented achievements have occurred in our understanding of fungal-host interactions, but the information is still fragmentary and limited to a few fungal pathogens. We have learned that insights gained from studies on one fungal disease do not necessarily apply to other fungal diseases. Notable insights have been gained as a result of work on candidiasis, cryptococcosis, histoplasmosis, blastomycosis, coccidioidomycosis, and paracoccidioidomycosis. Candidate virulence factors have been defined for a few of the etiologic agents. Examples are the phosphomannan complex on *Candida albicans*, phospholipase activity of *C. albicans* and *Aspergillus fumigatus*, a 120 kDa protein (WI-1) of *Blastomyces dermatitidis*, an hsp60 from *Histoplasma capsulatum*, α-1,3-glucan of *H. capsulatum* and *Paracoccidioides brasiliensis*, and pH regulation of macrophage phagolysosomes by *H. capsulatum*.

Studies on T cell immunity have identified T cell subsets and specific cytokines that are important in host defense against several of these fungal diseases. Depending on the disease, IFNγ, IL-12, IL-3, and CSF appear most significant in disease control. Furthermore, an abundance of data support a reevaluation of the importance of antibodies in host defense. The mere presence of antibodies in sera of patients who succumb to invasive disease is not sufficient evidence for an argument

against a protective role for antibodies. In fact, antibody specific for glucuronoxylomannan can be protective against cryptococcosis, and certain antimannan responses protect against experimental candidiasis. An important point is that typical antibody responses against whole fungal cells may not produce sufficient levels of protective antibodies, and new methods to enhance specific antibody responses are needed.

Antibodies are also in use for development of sensitive and specific immunoassays for diagnosis. These tests offer an alternative to the conventional culture methods that are slow and often of low sensitivity in cases of invasive disease. Recent progress includes a latex agglutination test that detects cryptococcal capsular polysaccharide in clinical specimens; *Candida* mannan assays that are sensitive to 0.2 ng mannan/ml serum; detection of fungal enolase and D-arabinitol as evidence for disseminated candidiasis; detection of galactomannan in the diagnosis of invasive aspergillosis; and reactivity with anti-WI-1 as evidence of *B. dermatitidis* infection. Furthermore, a *C. immitis* peptide was identified that may facilitate the monitoring of coccidioidomycosis patients for antibodies that have prognostic significance. In general, further immunological studies are needed for the development and implementation of preventive, therapeutic, and diagnostic approaches to fungal diseases.

Parasites

Although parasitic diseases are not a major cause of morbidity or mortality in the developed countries, they inflict enormous damage in the developing parts of the world (Table 13-1). The large differences in incidence among countries based on economic development apply to infectious agents in general. The percentages of total deaths caused by infectious agents in the developed and developing world are 1 percent and 43 percent, respectively. In most affected individuals, parasitic diseases are chronic and debilitating rather than life-threatening. An exception is malaria, which is the seventh leading cause of global deaths, killing 1.5 to 2.7 million persons annually. No vaccines against parasitic infections are currently in general use.

Parasites include diverse organisms whose fundamental biology, host/parasite interactions, and specific immune host defense mechanisms vary considerably. The helminthic parasites are multicellular, often long-lived organisms that generally lack the capacity to propagate intracellularly in the infected host. Thus, they often reside in extracellular sites, such as the vasculature or the gastrointestinal lumen. In contrast, protozoan parasites are unicellular organisms that replicate by varied and often complex means directly in the host and, depending on their species, may invade and survive within host cells. Studies of immune responses to parasites in humans and experimental animals have helped define the Th1 and Th2 subsets of helper T cells and have contributed to understanding the roles of these subsets and their cytokine products. It was found that responses to various parasites are marked by a predominance of Th1 or Th2 cells. While initial studies in specific strains of mice, especially with the protozoan *Leishmania*, suggested that Th1 cells are protective against intracellular pathogens and Th2 cells are protective against extracellular parasites, more

Table 13-1. Global Estimates of Common Parasitic Disease Incidence (1996)

Parasitic Disease	Deaths (thousands)	Prevalence (millions)
Malaria	1,500-2,700	>300
Trypanosomiasis	150	0.3
Leishmaniasis	80	3.8
Amoebiasis	70	N/A
Hookworm	65	151
Roundworm	60	250
Schistosomiasis	20	200
Lymphatic filariasis	N/A	119

N/A: not available
Source: World Health Organization, *World Health Report*, 1997.

recent observations indicate that protective responses are not so simply ascribed solely to specific T subsets.

As a consequence of their frequently obligate dependence for survival on the environment of the infected host, parasites have evolved in concert with mammalian hosts. Depending in part on the temporal and developmental stages of the parasites in the infected host and on the organs and cells involved, parasites use diverse mechanisms to regulate innate and adaptive immunity. With the widespread prevalence of many parasites as human pathogens, ongoing efforts to develop effective immunization protocols merit active pursuit. Many parasites, however, characteristically evade naturally acquired immune-based elimination, and rational approaches to identifying candidate immunogens and routes of immunization are currently very limited. The difficulties of initiating effective immune protection against parasites and the multiple biological interactions between the parasite and host emphasize the need to continue to define complex host/parasite interactions. These efforts include studies of the molecular mechanisms whereby parasites interact with target host cells, including mechanisms of attachment, cell invasion and intracellular survival, and, for multicellular parasites, mechanisms of modulation of host cell responses. The global prevalence of parasitic infections, including protozoan parasites that cause complicating and refractory opportunistic infections in patients with AIDS, further dictates that the efforts to identify immunopathogenesis and effective host immune responses be vigorously pursued.

Animal Models of Infectious Disease

Small Animals

Small laboratory animal models, particularly laboratory mice, provide valuable insight into infectious disease and host immune responses. Previous advances

have relied on microbiologically and genetically defined inbred mice, with a variety of naturally occurring genetic mutants, and this past decade has been heralded by large-scale and rapidly expanding application of molecular genetic techniques to the murine genome to create unique mouse strains. Thus, mouse biology will continue to be inextricably linked with biomedical research, regardless of the field of inquiry, in the coming decade. Utilization of homology relationships between human and murine genes, their products, and their associated pathophysiologic effects has been highly rewarding. In the next decade, clarifying the function of genes needed for the orchestration of effective immune responses to pathogens will be limited only by the number and type of mutations available for analysis. Further development of integrative genetic technology in the mouse will no doubt evolve into applications in humans. While targeted mutagenesis can test the effects of individual genes, discovery of novel and complex gene pathways can be more efficiently approached by large-scale mutagenesis combined with accurate phenotypic screening and analysis of the effects of infectious agents. Such models will serve to help characterize the functions of the many new human gene sequences that will soon emerge from the Human Genome Project.

In many cases, HIV/AIDS, for example, humans and mice are sufficiently diverse to prevent effective modeling of host-agent interactions. Nevertheless, mice engineered with human genes might allow future construction of useful models for HIV/AIDS. Furthermore, the mouse maintains a pivotal role in understanding basic immunology, allowing extrapolation to more complex models. The need remains for discovery and exploitation of other small laboratory animal models, particularly for diseases of childhood and persistent infections for which useful models currently do not exist.

Nonhuman Primates

Differences in the susceptibilities of mice and humans to various pathogens, and differences in their immune response mechanisms, impose significant limitations on the direct applicability of mouse research to human disease. Such differences can be minimized by using as models our close genetic relations, the nonhuman primates. Although these animals are less well characterized for some aspects of immunity than are mice, and large animal research imposes considerably greater costs, the use of nonhuman primates for preclinical studies has already proved to be valuable. A partial list of pathogens that infect both nonhuman primates and humans is presented in Table 13-2. Fungi are omitted from the table, since fungal diseases are not common in nonhuman primates. However, representatives of all the major human mycotic pathogens have been found in more than one nonhuman primate species, and they are transmissible to humans. Fungi such as *Histoplasma capsulatum*, *Coccidioides immitis,* and several *Aspergillus* species cause systemic infections and chronic or acute granulomatous or suppurative lesions in the respiratory tract or bone. *Cryptococcus neoformans* may cause granulomatous pulmonary and cerebral lesions, and *Candida albicans* may cause localized thrush on the oral or genital mucosal surfaces of neonates or a disseminated disease in

Table 13-2. Nonhuman Primate Susceptibility to Infectious Agents

Infectious Agent	Affected Host(s)
Bacterial Infections	
Shigella sp.	Great apes, macaques, New World monkeys, baboons, other African species
Salmonella sp.	South American and Old World monkeys, apes
Mycobacterium tuberculosis	All nonhuman primates
Mycobacterium leprae (leprosy)	Sooty mangabeys, chimpanzees
Viral Infections	
Human immunodeficiency virus	Chimpanzees, macaques
Respiratory syncytial virus	Chimpanzees
Herpesvirus hominis (herpes simplex)	Ringtail lemurs, patas monkeys, tree shrews, owl monkeys, marmosets, gorillas, gibbons
Parainfluenza (myxovirus)	Patas monkeys, chimpanzees, marmosets, gibbons, cynomolgous macaques, vervets, capuchins, baboons
Hepatitis A	Chimpanzees, cynomolgous macaques, woolly monkeys, celebes macaques, siamangs, owl monkeys, patas monkeys
Hepatitis B and C	Chimpanzees, orangutans, gorillas
Parasitic Infections	
Entamoeba histolytica	Old World monkeys
Enterobius vermicularis (pinworms)	Old World primates and great apes
Leishmania sp.	Old World monkeys
Schistosoma sp.	Baboons
Filariae	Old World monkeys

Source: D. Rick Lee, D.V.M.

Research Opportunities

Immune Activation by Pathogens

- Further define antigen processing and presentation pathways to determine differences among antigen-presenting cell types and conditions that alter effective presentation of infectious pathogens at different stages in their life cycles
- Determine methods to predict the peptide epitopes of pathogens that are most effective for T and B cell stimulation and immune protection
- Identify the APC that are most effective at generating memory T and B cells and develop methods to target antigens to appropriate APC and appropriate processing compartments to optimize vaccine efficacy in promoting immune memory
- Clarify the role of antibodies in host defense against fungal and parasitic diseases

Mechanisms of Pathogenesis

- Accelerate sequencing projects to identify bacterial and viral genes important in human pathogenesis
- Identify microbial products that interfere with protective immune responses
- Identify mechanisms that control and regulate fungal cell wall synthesis and organization
- Elucidate pathogenic mechanisms of non-*albicans Candida* spp., *Aspergillus* spp., and the dematiaceous (black) yeasts

Animal Models of Pathogenesis and Immune Protection

- Utilize transgenic/knockout mice, specific immunosuppressive or immuno-activating agents, and clinical studies to develop novel antimicrobial drugs and vaccines
- Develop and characterize animal models, including nonhuman primates, that accurately mimic human infectious diseases

Human Research in Infection and Immunity

- Develop improved, sensitive diagnostic tests for invasive infectious disease for use as prognostic indicators to monitor progress of patients during antimicrobial therapy
- Define molecular mechanisms of interactions between intestinal mucosal cells, the mucosal immune system, and prevalent protozoan infections of the alimentary tract, such as cryptosporidiosis
- Characterize elements of the human innate and adaptive immune systems that contribute to the balanced survival of host and parasite in conditions of persistent infections
- Continue studies on the life cycles of parasites in the human host to assist in vaccine development
- Develop new analytical tools to monitor infection, pathogenesis, and immune reactivity *in vivo*

immunocompromised animals. Ringworm and athlete's foot, caused by *Microsporum* and *Trichophyton*, respectively, have also been reported in nonhuman primates.

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Novel Strategies for Vaccine Development

14

Overview

Successful vaccines are enormously powerful weapons against infectious diseases, and widespread immunization can dramatically reduce disease incidence, as has been accomplished in the United States for diphtheria, measles, tetanus, whooping cough, and mumps. Furthermore, worldwide vaccination has led to the global elimination of smallpox, and there is hope that polio will soon be eradicated in all nations. Unfortunately, not all vaccines are as successful as these in preventing infections, and very few therapeutic vaccines are available that can halt disease after infection occurs. The association of liver, stomach, cervical, and other cancers with chronic infections further highlights the need for improved vaccine strategies to prevent or reverse infection by a variety of debilitating or lethal microorganisms.

The extraordinary variety of bacteria, viruses, and parasites that continue to threaten human health poses a distinct challenge to both basic and clinical immunologists, and the field of vaccine research has recently undergone a revolutionary change. In the past, most vaccines were developed empirically, whereas current approaches take advantage of the enormous recent expansion of knowledge in basic immunology and molecular biology. Specific components of the immune system judged to be the most effective against particular organisms are being targeted with novel immunization strategies. Innovative approaches are likely to be critical for the development of effective vaccines against pathogens that have defeated previous efforts, such as respiratory syncytial virus, pathogens for which current vaccines are inadequate, such as *Mycobacterium tuberculosis*, and pathogens for which there are as yet no vaccines, such as HIV-1. A representative list of diseases for which vaccines are needed is given in Table 14-1.

Targeting Specific Elements of the Immune Response

A critical requirement of successful vaccination is the induction of immune responses that are durable and/or boostable. Thus, vaccines should induce long-lasting immune memory (Chapter 4), and different vaccine modalities will have varied abilities to generate robust immune memory. The rational design of vaccines also depends on basic knowledge of the particular immune mechanisms that are the most important in combating the infection. Effective mechanisms can be identified experimentally by the transfer of antibodies (immune globulin) or immune cell populations, or by the selective ablation of immune effector pathways. In humans, the ability to transfer immune globulin passively has allowed definitive roles to be assigned to antibody-mediated mechanisms for bacterial diseases such as the bacteremia and meningitis caused by *Haemophilus influenzae* type b (Hib) and *Streptococcus pneumoniae*. For cellular immune mechanisms mediated by T cells, natural killer (NK) cells, monocytes, and neutrophils, animal

Table 14-1. Need for Vaccines

DISEASE	ANNUAL DEATHS	PERCENT TOTAL DEATHS	ANNUAL NEW CASES
Diarrheal Diseases	2.5-4 million[1,3]	4.8-7.7%	1.3 billion[3]
Acute Respiratory Diseases	3.7 million[1]	7.1 %	
Tuberculosis	2-3 million[2]	3.8-5.7%	7-8 million[2]
HIV	2.3 million[4]	4.4%	5.8 million[4]
Malaria	1.5-2.7 million[5]	2.9-5.2%	300-500 million[5]
HPV (cervical cancer)	> 300 thousand[6]	0.6%	> 500 thousand[6]

[1]http://www.who.ch/whr/1998/presse.htm
[2]http://www.who.ch/gtb/publications/factsheet/index.htm
[3]http://www.who.ch/chd/pub/cdd/meded/1med.htm
[4]http://www.unaids.org/highband/document/epidemio/report97.html#top
[5]http://www.who.ch/inf/pr/1997/97wha1.html
[6]http://www/who.ch/press/1996/pr96-47.html

models and rare primary immune deficiencies in which selected effector systems are impaired have provided valuable information.

Antibody Responses

Although antibody transfer provides only transient protection, experimental transfer studies are useful in defining the value of antibody-mediated protection against a particular pathogen. Antibody neutralization is the only known mechanism capable of completely preventing infection by a pathogen, a condition referred to as sterilizing immunity. In addition to neutralization, an efficient and prolonged antibody response also protects by mediating the lysis and opsonization of pathogens. These three effector functions—neutralization, lysis, and opsonization—can result directly from antibody binding to a pathogen or indirectly from antigen-antibody (Ag:Ab) complex-mediated activation of the classical or alternative pathways of the complement system.

Passive transfer of immune globulin to children at high risk for invasive Hib disease provides significant protection against bacteremia and meningitis. Transfer of bacterial polysaccharide-reactive immune globulin to children at risk for pneumococcal invasive disease also protects. In both examples, antibody effector function is mediated by complement. Antibody plus complement is directly bactericidal for Hib, while antibody-mediated deposition of complement products onto the pneumococcal bacteria causes phagocytosis and killing by neutrophils. Antibodies that neutralize bacterial toxins, such as the toxins of *Clostridium tetani* and *Corynebacterium diphtheriae*, were widely used for treatment before antibiotics became available. Antibodies to *Bordetella pertussis* toxin protect in animal models, and direct

immunization with pertussis toxin was shown to protect against whooping cough in a clinical study. In the case of viral infections, passively administered immune globulin or monoclonal antibodies can protect against respiratory syncytial virus and the hepatitis A and B viruses (HAV and HBV) in humans. In many animal studies, passive transfer of specific neutralizing antibodies was shown to prevent subsequent infection by numerous viruses including influenza, HIV, and papillomavirus.

Cell-Mediated Responses

Several different types of cell-mediated immunity play critical roles in responses to bacterial and viral pathogens. The role of neutrophils in defense against bacteria has been demonstrated both *in vitro* and in clinical studies of patients with primary defects in neutrophil function, who suffer multiple infections with bacteria. In many cases, antibodies to the bacteria mediate uptake and killing by neutrophils. Assessment of the role of macrophages *in vivo* is complicated by the intimate interactions between macrophages and T lymphocytes in adaptive immunity. Macrophages phagocytose microorganisms but generally require activation by cytokines from T or NK cells to produce reactive oxygen intermediates that then kill the intracellular pathogen. Thus, defense against *Listeria monocytogenes*, *Mycobacterium tuberculosis*, and *Leishmania*, for example, is dependent on both macrophages and T cells. Effector functions of CD4 T cells are mediated principally through the production of cytokines, such as IL-2, IL-4, IFNγ, and TNFα, that activate macrophages to kill intracellular pathogens and activate B cells for antibody production.

The major function of CD8 T cells and NK cells is to kill infected cells directly, but they also produce cytokines that may have critical effector functions. For example, IFNγ produced by NK, CD4, and CD8 cells is important in responses to intracellular bacteria, fungi, protozoa, and viruses such as influenza, cytomegalovirus (CMV), vaccinia, and herpes simplex (HSV). Patients with NK cell deficiencies experience very severe initial herpesvirus infections, suggesting that NK cells may be a principal means of limiting infection before specific adaptive immunity develops. An increasing body of evidence suggests that CD8 cytolytic T cells (CTL) are critical components of the response to certain viruses such as HIV-1 and CMV. During the past decade, advances in understanding the basic mechanisms of CTL induction in mice have spurred recent efforts to design human vaccines that specifically generate CTL responses. However, significant progress will depend on filling the glaring gaps that exist in understanding human CTL responses. With the possible exception of work with HIV-1, there is currently little systematic information on the nature, extent, or duration of human CTL responses to infection or vaccination, and this deficit is a major stumbling block to rational vaccine design. In some cases, the induction of CTL may not be desirable, because CTL might produce injury in uninfected bystander tissues. Therefore, the most effective type of immune response for a particular pathogen must be determined to elicit the most appropriate response to vaccination.

Antigen-Presenting Cells

Targeting Antigens to Follicular Dendritic Cells for Antibody Responses

The generation of effective antibody (Ab) protection requires the deposition and persistence of foreign antigen within the germinal centers of lymphoid tissue, the sites where antibody-producing B cells are formed. Antigens bind to the surfaces of highly specialized antigen-presenting cells (APC), called follicular dendritic cells (FDC), and can be retained within lymphoid tissue for years. FDC are a small population of cells within germinal centers, and they are unique APC that play a major role in the maintenance of immune memory by continually presenting antigen to B cells. FDC actually bind Ag:Ab complexes on long cytoplasmic extensions that form a meshwork throughout the lymphoid follicle. Unlike macrophages and other "professional" APC, FDC do not ingest this membrane-bound antigen complex. Rather, it is retained in an undegraded form that is effectively recognized by B cell receptors, internalized by the B cells, and then presented to neighboring T cells to activate cytokine secretion, antibody production, and continued B cell memory. The molecular mechanisms that generate Ag:Ab:FDC complexes have not been fully elucidated, but a likely model has been devised based on current data. Following immunization, initial antibody production and Ag:Ab complex formation, the complex activates the complement system to generate the complement component, C3b, which attaches covalently to the Ag:Ab complex. Then, Ag:Ab:C3b complexes bind to FDC receptors specific for C3b or for the Fc domain of the antibody (FcR). The undegraded antigen now retained by the FDC provides the stimulus for germinal center formation and consequent B cell differentiation events. Very small quantities of antigen within this FDC depot can perpetuate memory B cells and maintain a prolonged and effective antibody response without fresh administration of antigen.

Based on these findings, one promising strategy for vaccine development is to target antigens directly to FDC, and one future approach is to couple antigens to FDC-specific monoclonal antibodies for immunization. As yet, few FDC-specific target molecules are known, but a human FDC-specific protein was recently identified and antibodies reactive with this protein have been produced. A second approach is to immunize with preformed Ag:Ab complexes capable of immediate complement activation and FDC targeting, without the need for initial B cell activation. A third approach is to eliminate the need for antigen-specific antibodies, by creating fusion proteins composed of the foreign antigen directly coupled to complement ligands that can bind to FDC receptors. This strategy was recently used in mice, and it was found that fusion of C3b to a test antigen increased its immunogenicity for antibody production by 10,000-fold. Such remarkable results offer great promise for future applications of this strategy.

Enhancing T Cell Responses to APC by Local Cytokine Delivery

Antigen presentation to T lymphocytes is most effectively accomplished by a completely distinct lineage of APC, the blood-derived dendritic cells (DC), and recent efforts to increase tumor immunogenicity

have led to a strategy for enhancing antigen presentation by DC. First, the role of individual cytokines in enhancing vaccine-induced T cell responses was studied using tumor cells transduced with various cytokine genes to generate high concentrations of the cytokine in the vicinity of the tumor. It was found that granulocyte-macrophage colony-stimulating factor (GM-CSF)-transduced tumor cells induced potent tumor immunity. The impressive effect of GM-CSF may result from its unique ability to promote the differentiation of immature precursor cells into mature DC. Efficient T cell activation requires costimulatory signals in addition to engagement of the T cell antigen receptor, and such costimulatory signals are abundant on mature but not immature DC. Thus, local GM-CSF production may facilitate the rapid production of mature DC, which can then present antigens shed from the tumor cells. This cytokine transduction approach is now being tested in experimental vaccines for infectious pathogens, with encouraging results in some model systems.

Targeting Antigen Processing Compartments With Particulate Antigens

The most frequent types of vaccine in clinical use are inactivated pathogens or their subcomponents because these non-living preparations have a long history of safe use in humans. However, there are several diseases for which this type of vaccine is suboptimal or ineffective. One major limitation is that non-living vaccines generally do not stimulate CTL, which are required for protective responses against many cancers and viral infections. CTL are now known to be activated by MHC class I molecules that bind antigenic peptides derived from intracellular proteins; the peptides must be generated intracellularly in APC for efficient presentation to CD8 T cells. Although natural infection will produce intracellular viral peptides, inactivated or subunit vaccine antigens fail to enter the appropriate cytoplasmic compartment and are unable to stimulate CTL immunity. Based on this understanding, recent work has demonstrated that isolated antigens can be conjugated to particles that target them into the cytoplasm for normal presentation on MHC class I molecules. Importantly, this process occurs most efficiently in precisely those cells that are critical for initiating T cell responses: DC and macrophages. This approach is applicable to many different antigens, and injection of such particulate antigen preparations into animals has been shown to prime for both CTL and CD4 helper T cell responses. Several features of this approach are attractive for translation to clinical situations. In the preclinical mouse models, effective vaccination is obtained by a simple subcutaneous immunization in saline; thus, inflammatory adjuvants, which may be deleterious in humans, are not required. In addition, strong immunity is elicited with a single injection.

Novel Vaccine Delivery Systems

Vaccine delivery was a relatively neglected area of research until it was realized that the immune response can be greatly influenced by the way in which an antigen is presented to the host. One area of particular interest is the use of recombinant DNA technology with live bacterial or viral vectors to express the relevant antigens in the host. Although the potential choice of vector microorganisms is large, it is limited to those that infect

humans without causing clinical illness. Attenuated vaccines that are currently in use, such as vaccinia and BCG, are preferred because they are already accepted by licensing authorities. Some attenuated vectors, such as adenovirus, the Sabin polioviruses, and *Salmonella typhimurium*, can be used to deliver antigens orally. Furthermore, some bacteria, such as BCG, *Salmonella*, and *Listeria*, and viruses like vaccinia, can induce CTL responses. However, live microbial vectors are themselves immunogenic, and the resulting immune response severely restricts repeated immunization with the same vector. In addition, live vectors may cause disease in immunocompromised individuals. Difficulties may be encountered, even in the initial immunization, due to preexisting immunity in the general population against most of the currently studied vectors, such as attenuated *Salmonella* and polio and influenza viruses. Furthermore, mere colonization with recombinant bacteria is usually not sufficient to evoke vigorous responses. Strains causing even transient bacteremia are far more effective, and this requirement is likely to be of concern to vaccine regulatory agencies. In addition, the expression level of foreign antigens is usually low and can be overshadowed by vector antigenicity, and the structure of the antigen may differ from that found in the native pathogen, thus limiting effectiveness. Live vectors present serious safety issues in immunocompromised individuals. This is a considerable problem in developing countries where prior determination of HIV-1 status is not feasible. Efforts to attenuate live vectors are under way, but immunogenicity might be affected, and there is a clear need to develop novel vectors that attain an optimal balance between safety and immunogenicity.

A new method for delivering antigens that circumvents some of these problems was described in the early 1990s. Remarkably, it was found that injection of expression plasmid DNA that encodes an inserted protein antigen can induce protective immunity against challenge with the pathogen expressing that antigen. Positive results have been demonstrated in a variety of preclinical models, and such noninfectious "DNA vaccines" are now an area of intensive research. DNA vaccines offer a number of potential advantages that might eliminate the shortcomings of live or subunit vaccines. However, this approach would not generate antibodies against a sugar capsule, which may be needed for protection against certain bacteria. Furthermore, it is possible that successful human DNA vaccination might require large amounts of vector, multiple immunizations, or protein or subunit boosting to achieve fully protective immunity. Clearly, adjuvant or delivery systems that can increase the potency of DNA vaccines will be very useful, and novel technologies for targeted delivery may also be required. One general strategy under investigation is the construction of "genetic adjuvants," which codeliver DNA encoding certain cytokine or other immunostimulatory sequences together with the antigen sequence. It is already known that the vector DNA itself provides some adjuvant activity.

Mucosal Vaccines

Mucosal immunity is of considerable importance in vaccine development because (1) nearly all viral, bacterial, and parasitic agents that cause diseases of the intestinal, respiratory, and genital tracts enter through mucosal membranes;

(2) mucosal and systemic immune responses are often elicited and regulated independently, and induction of protective immunity at the most frequent sites of entry is likely to be most effective; (3) young children and elderly individuals may respond better to mucosal vaccines because the mucosal immune system develops earlier and appears to remain functional longer than the systemic compartment; and (4) mucosal immunizations are simpler and less expensive than systemic immunizations; for example, the existence of an oral polio vaccine has allowed immunization campaigns that may soon eradicate polio worldwide. The disadvantages of some current mucosal vaccines include (1) the short duration of immune responses and necessity for frequent reimmunization; (2) low absorption of antigens from mucosal surfaces and great variability of absorbed doses among individuals; and (3) the possibility of inducing T cell unresponsiveness to some of the vaccine components because oral antigen administration often leads to tolerance in experimental systems.

Several strategies for the development of improved mucosal vaccines are being pursued. One attractive approach is the use of microorganisms that naturally colonize mucosal tissues as vectors to introduce an antigen gene from an unrelated microorganism. Although reported results have been encouraging, there are considerable difficulties with the general applicability of such delivery systems, as discussed above. Other strategies under study include the introduction of microbial antigen genes into edible plants, such as tomatoes, bananas, spinach, and potatoes. This approach represents a potentially important strategy for the production of inexpensive oral vaccines, particularly useful in developing countries. Although initial results with such vaccines in animal models are encouraging, antigen expression levels must be increased before this approach will be useful. As alternative approaches, candidate antigens have been incorporated into liposomes, biodegradable particles, cochleates, or immunostimulating complex matrix (ISCOM) preparations. In general, such particulate antigens are absorbed from mucosae better than soluble forms, and in some systems, the incorporated antigens are protected from gastric and intestinal degradation. Nonetheless, a dishearteningly small fraction of the ingested particles is absorbed in an immunogenic form.

The observation that enterotoxins such as cholera toxin (CT) and the heat-labile toxin (LT) of *Escherichia coli* are potent mucosal immunogens has led to their use as antigen carriers or as adjuvants coadministered with other antigens in experimental systems. As carriers, the nontoxic binding (B) subunits have been covalently coupled to a variety of protein or carbohydrate antigens. Oral or intranasal immunization induces high levels of both mucosal and systemic antibody responses that can persist for prolonged periods. Although the extreme toxicity of native CT and LT precludes their use as adjuvants in humans, several recent studies have reported that nontoxic mutants retain adjuvant activity in experimental animals. Further studies are now required to evaluate these mutants as adjuvants, to determine the immunogenic properties of antigens coupled to CTB or LTB, to determine whether CTL and long-lasting memory responses can be generated, and

to eliminate tolerogenic potential from such preparations.

Adjuvants

Increased recent interest in adjuvant research has been driven by the availability of recombinant subunit vaccines. Because these highly purified protein vaccines lack the microbial components that trigger nonspecific immunostimulation, their inherent immunogenicity is much lower than that of traditional live, attenuated, or even inactivated whole pathogen vaccines. Many of these subunit vaccines may not be useful without adjuvants more potent than alum, the only adjuvant currently licensed for human use. Other known adjuvants are associated with significant toxicity, and the major goal in this area is to identify safe yet highly potent immunostimulatory compounds that will increase antigenicity when combined with vaccine antigens.

Several promising candidates are in late clinical testing. These include MF59 (a stable emulsion consisting of metabolizable oil and surfactants), monophosphoryl lipid A (MPL), polyphosphazene, virosomes (viral proteins in lipid vesicles), and soluble (QS21) and particulate (ISCOM) forms of purified saponins from the Quillaja saponaria tree. MF59 has been used in clinical trials with HSV, HIV-1, CMV, influenza, and HBV subunit vaccines in more than 8,000 people. These studies have established an excellent safety record and have demonstrated that the adjuvant is very potent in stimulating human antibody and helper T cell responses. MF59 is now part of a licensed influenza vaccine in Europe. MPL has been used in clinical trials with cancer, malaria, HSV, HBV, and HIV-1 vaccines without significant adverse effects. QS21 has been used in clinical trials with cancer and HIV-1 vaccines. Combinations of MPL and QS21 have been tested with HBV, HSV, influenza, and malaria vaccines, and this mixture stimulated very good immune responses and protection in a malaria challenge study. Polyphosphazene and ISCOM adjuvants are currently in clinical trials with influenza vaccine. A hepatitis A vaccine using influenza-containing virosomes as adjuvant was recently licensed in Switzerland. In general, these new adjuvants appear to be safe enough for human vaccine use, and they induce much better antibody and cellular immune responses than alum. They also appear to be economically useful for widespread vaccination. However, a number of issues still need to be addressed.

Most of the adjuvant systems currently in development are effective only in the priming phase of the immune response. A practical example is influenza vaccination. Most people are already primed to influenza from natural infection or previous vaccination. In such cases, existing adjuvants provide only a modest improvement in the response to influenza vaccine. Thus, additional research is needed to define useful mechanisms for boosting responses in pre-primed situations. Mucosal delivery of vaccines presents a number of technical difficulties, including the need for specialized adjuvants and vaccine delivery vehicles. Further research is needed to define the best antigen formulations and adjuvants and to understand the importance of secretory IgA in protective immunity. The inefficient induction of CTL responses by non-living vaccines also presents a special problem.

Although subunit vaccines combined with a number of different adjuvants or delivery vehicles can induce potent CTL responses in mice, strong and durable CTL responses in primates and humans have not been achieved. One additional problem is that the duration of protective immunity is often limited, suggesting an inability to induce sustained immune memory. Thus, subunit vaccination often requires multiple immunizations, resulting in regimen compliance problems with pediatric recipients and in developing countries. Clearly, adjuvant systems that provide protective immunity with a single immunization should be sought.

Vaccines for Children and the Elderly

Protective immunity is often difficult to achieve in either very young or aged individuals, because their immune systems differ from the adult system in ways that are not yet well defined. Vaccines for pathogens that infect the newborn, such as group B streptococcus, *E. coli, Listeria*, and *Staphylococcus*, present special challenges. Current medical practice includes antibiotic treatment of infected neonates or of colonized mothers prior to delivery. Alternative strategies could include maternal vaccination or passive transfer of immune globulin to infants at risk. Pediatric immunization would benefit from the development of new combination vaccines to simplify administration, improve compliance, and broaden the scope of protection. Currently, childhood vaccination in the United States requires as many as 18 separate doctor visits. The major objective of combination vaccines is to induce comparable protective immunity to each of the individual components. For some combinations, it is known that dominant responses to certain epitopes preclude vigorous responses to the other components. Furthermore, some antigen sequences can be immunosuppressive. New adjuvant formulations may eliminate some of these problems, but alternate recombinant antigens may be required in some cases.

Serious gaps exist in the efficacy of current vaccines for clinically important bacterial pathogens in children, including the major otitis media-causing pathogens, *S. pneumoniae*, nontypeable *H. influenzae*, and *Moraxella*. The current 23-valent carbohydrate vaccine for *S. pneumoniae*, which is the primary cause of pneumonia and otitis media in children, provides inadequate coverage for infants due to their poor immune responsiveness to carbohydrate antigens. Newer generations of this vaccine include the six or seven most prevalent serotypes, conjugated to protein carriers to enhance immunogenicity, but it is not yet clear whether this approach will be effective for most serotypes. Additional strategies include identification of the most immunogenic, conserved proteins among the various serotypes utilizing the newly sequenced genome of *S. pneumoniae*. *Neisseria meningitidis* also poses a special problem, because the meningococcus carbohydrate antigens stimulate cross-reactive pathogenic responses in the central nervous system, thus precluding full coverage against the group B serotypes. Genomic sequencing and empiric screening of the proteins derived from putative open reading frames could lead to the identification of new vaccine candidates. The importance of mucosal secretory IgA responses needs to be evaluated for pathogens such as *S. pneumoniae* and nontypeable *H. influenzae*

that infect the respiratory tract. IgA responses have a presumed advantage, but systemic vaccination or passive transfer of immune IgG has been sufficient to reduce colonization or prevent disease by *H. influenzae* type b or respiratory syncytial virus.

The adolescent and young adult community has not been a major focus of efforts to develop new bacterial vaccines. However, opportunities exist for both prophylactic and therapeutic vaccines to prevent common sexually transmitted diseases such as *Neisseria gonorrheae*, Herpes simplex virus, human papillomavirus, and *Chlamydia trachomatis*; recurrent infections in sexually active individuals such as uropathogenic *E. coli*; and *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* in all age groups.

There is a growing need to provide more adequate vaccine coverage for pathogens affecting the elderly, because the geriatric population in the United States continues to expand and life expectancies continue to increase. Vaccines that are effective in younger adults are often ineffective in the elderly due to depressed immune responses. Furthermore, certain bacterial infections occur more frequently in patients with underlying chronic disorders associated with aging, such as diabetes, bladder stasis, or valvular heart disease, or in those who frequent hospitals regularly and are at risk of nosocomial infections. Diminished immunity is a well-recognized feature of the aging process. For example, hepatitis B, tetanus, and influenza vaccines are often ineffective, and both antibody and CTL responses to influenza are reduced in the elderly. It is less clear whether antibody responses to T-independent antigens such as pneumococcal polysaccharide are reduced. There are a variety of infections currently targeted for vaccine development, and approaches should be optimized to induce protection at all stages of life: childhood, adult, and elderly. In this regard, the recent development of an effective nasal spray vaccine for pediatric influenza might serve as a good example. A novel temperature-sensitive influenza vaccine was produced that mimics the first stages of flu infection by replicating in the relatively cool passages of the nose and inducing specific immune responses. However, the vaccine does not cause disease because a mutation prevents its replication in the much warmer lower respiratory tissues. This truly novel vaccine was shown to protect children aged 15 months to 6 years, and it will now be tested in other age groups, including the elderly. Similar approaches could be used for other mucosal and perhaps systemic pathogens.

Although a variety of new approaches are available that might produce effective vaccines for the elderly, further research is needed to understand the causes of diminished immunity with aging. The most evident immunological change accompanying aging is thymic involution, resulting in a decreased rate of new T cell generation and fewer naive T cells in the periphery. Changes in mature T cell populations also include decreased numbers of CD8 cells, CD4 responses dominated by Th2 rather than Th1 cytokines, and decreased proliferative responses to IL-2. Alterations in B cell function include a shift of antibody responses toward IgM, decreased antibody affinities, a restriction

of the B cell repertoire, and an increase in circulating autoantibodies. Although not entirely resolved, certain immunological functions appear to be intact, such as antigen presentation, macrophage and neutrophil phagocytosis, and NK cell function. Despite these preliminary advances in understanding the aged immune system, the specific mechanisms that prevent effective immunization in the elderly are not well defined, and immunosenescence is a fertile area for intensified research efforts.

Nonhuman Primate Models for Vaccine Research

Nonhuman primates are most similar to humans genetically, anatomically, physiologically, and immunologically and are therefore the most relevant animal models of human immunity. Although most similar to humans, chimpanzees are not ideal for routine vaccine studies due to limited numbers of animals and prohibitively high costs. Since the formation of the Regional Primate Research Centers by the National Institutes of Health in the 1960s, several macaque species have been used extensively in biomedical research. These species are well suited to vaccine research as they are readily available and breed domestically. Humans and macaques have striking similarities in reproductive anatomy and development, with features unlike those of rodents, carnivores, and ungulates. Recent studies have shown similarities in the human and macaque fetal and neonatal immune responses to viral infection and, in contrast to the rodent, macaque maternal antibodies are transferred transplacentally to the fetus during the latter part of gestation as in humans. Thus, the macaque neonate provides a very useful model for investigations of therapeutic strategies and the ontogeny of immune responses. The physiological and endocrine changes that occur during puberty and aging are also thought to be similar in macaques and humans. Unfortunately, the study of immune responses in nonhuman primates is still in its infancy, in part because many of the reagents needed for such work are not available. Although some antibody, cytokine, and molecular reagents developed for human studies are now known to react with macaque molecules, there are still significant gaps in the repertoire of reagents required for nonhuman primate research.

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Research Opportunities

Basic Immunology

- Expand basic immunology studies to define the types of immune responses that provide the most effective protection against specific pathogens
- Define the nature, extent, and duration of CTL activity in human responses to infection and vaccination
- Develop additional strategies to direct vaccine antigens to the most potent APC and to target appropriate processing pathways for MHC class I and II presentation
- Determine the requirements for inducing robust immune memory
- Apply basic findings to the development of new vaccines and the improvement of existing vaccines

Vaccine Delivery Systems

- Engineer novel live vaccine vectors that induce strong and durable responses to the inserted antigen and that are safe for immunodeficient individuals
- Further evaluate the efficacy of DNA vaccines and the effects of adjuvants on DNA immunization
- Determine the mechanisms by which DNA vaccines stimulate particular types of immune responses and the antigen presentation pathways utilized by DNA vaccine antigens

Mucosal Vaccines

- Compare various approaches for the induction of mucosal immune responses and determine the applicability of results obtained in animal models to humans
- Investigate the duration of mucosal immune responses, the possibility of inducing immune tolerance, and the variable absorption rates of antigens from different mucosal surfaces or between individuals

Adjuvants

- Define the biological functions of adjuvant components to develop more purified preparations and eliminate toxic side effects
- Develop adjuvants that promote durable immune responses after a single immunization
- Develop adjuvants that enhance the induction of different types of immune responses, including Th1 *versus* Th2 responses and different antibody isotypes
- Develop adjuvants capable of boosting responses in pre-primed individuals
- Identify specialized adjuvants for the induction of CTL responses; systematically study human CTL responses during infection and after immunization
- Determine optimal strategies for mucosal adjuvant development

Vaccine Development for Different Age Groups

- Characterize the mechanisms responsible for developmental changes in the human immune system, including fetal, neonatal, adolescent, adult, and elderly immunity
- Expand vaccine development for sexually transmitted diseases
- Identify the immunological basis for reduced responses to important vaccines in aged humans
- Identify approaches to optimize vaccines for infants and to restore effective vaccine responses in the elderly
- Develop markers to predict readily the efficacy of candidate vaccines for specific pathogens

Animal Models for Vaccine Development

- Develop macaque-reactive immunological reagents to facilitate studies on nonhuman primate vaccine models
- Study macaque immunoregulation to understand better the basis for responses in infected *versus* vaccinated animals, responses to adjuvant-antigen combinations, responses to mucosal *versus* parenteral immunization, and responses to novel antigen delivery methods
- Develop MHC haplotype-matched macaques to facilitate genetically controlled studies of vaccine efficacy

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Genetic Basis of Disease Susceptibility

15

Overview

There has been a recent explosion of important new information on the genetics of immunity and immune diseases. Human diseases can now be studied using new techniques, analytical methods, extensive databases, and varied resources that provide powerful new genetic approaches. These promise to transform our fundamental concepts of presently enigmatic disorders and provide more effective strategies for diagnosis, prevention, and therapy. Recent progress on the genetics of AIDS, diabetes, rheumatoid arthritis, systemic lupus erythematosus, hemochromatosis, ataxia telangiectasia, inflammatory bowel disease, asthma and allergic diseases, and multiple sclerosis, among other diseases, demonstrates the enormous potential of genetic studies to clarify disease etiology and suggest effective treatments. With the appropriate investment of human talent and financial resources, many opportunities exist to expand genetic understanding of human immunity and to alleviate suffering from infectious and immune-mediated diseases.

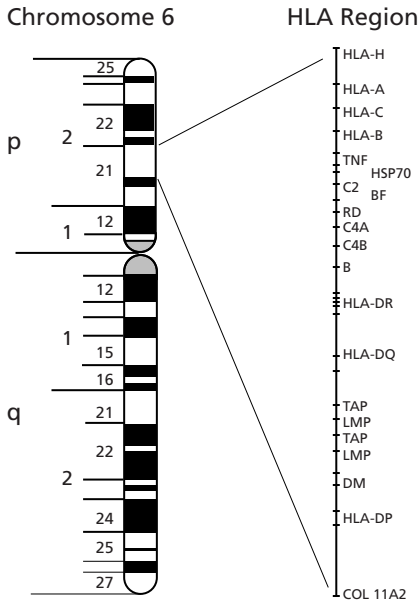
Known Genetic Loci

HLA

There are a number of classically known genetic loci associated with human disease. One of the most important for immune-related diseases is the major histocompatibility complex (MHC), which is called HLA in the human. The genes found in the HLA region determine the response to transplantation and contribute to the particular nature of other immune responses such as autoimmune susceptibility and survival after infection. The 3.5 megabases of the HLA DNA region are only a small part of chromosome 6 and about 0.1 percent of the entire human genome. Many of the genes found there are highly polymorphic, meaning that one person is unlikely to have an HLA gene identical to another randomly chosen person. Figure 15-1 shows a partial map of the HLA region and some of the many diseases that have been associated with HLA genes. The great majority of these diseases are inflammatory and are thought to involve the immune system, because many of the HLA genes encode proteins involved in antigen presentation to T cells. The MHC class I (HLA-A, B, C) and class II (HLA-DR, DP, DQ) proteins directly present antigenic peptides to CD8 and CD4 T cells, respectively. Extensive MHC polymorphism helps explain variations in disease susceptibility because different MHC sequences vary in their capacities to bind stimulatory antigens. Other HLA genes, such as TAP-1, TAP-2 and HLA-DM, are involved in the generation of peptide-MHC complexes.

One example of an important recent genetic advance concerns the iron storage disease, hemochromatosis. Previous work had established that hemochromatosis is associated with the HLA-A3 allele. Among people of northern European extraction, approximately 1 in 10 is

Figure 15-1. The HLA region of chromosome 6 contains susceptibility genes for many diseases.



HLA Region-Associated Diseases

Hemochromatosis
Psoriasis vulgaris
Uveitis
Reiter's syndrome
Ankylosing spondylitis
Systemic lupus erythematosus
Rheumatoid arthritis
Sjögren's syndrome
Dermatitis herpetiformis
Myasthenia gravis
Goodpasture's syndrome
Addison's disease
Graves' disease
Juvenile rheumatoid arthritis
Multiple sclerosis
Celiac disease
Type 1 diabetes
Narcolepsy

Source: John Harley, M.D., Oklahoma Medical Research Foundation, University of Oklahoma, 1997.

heterozygous for the hemochromatosis gene. It was not known whether the genetic effect is related directly to the A3 allele or whether some neighboring locus is responsible for the phenotype. Recent work has confirmed the latter interpretation. It was found that a mutation in a previously unknown class I gene (HLA-H) accounts for 85 percent of the northern Europeans with hemochromatosis. Neither the biological basis nor the mechanism by which this mutation causes hemochromatosis is now known, but any such understanding is almost certain to be built on this important genetic discovery and must involve this newly identified gene.

Such accomplishments have not occurred in a vacuum. They are made possible only by continued investment and progress in molecular biology and statistical genetics, by access to clones of DNA from particular areas of the human genome, by construction of massive databases containing all of the known DNA sequences, by computers capable of managing the data, and by software capable of extracting useful new information. The investments made previously in equipment, databases, and technical capability have been very productive and show every promise of continuing productivity. These strategies are being used by hundreds of investigators studying a variety of complex genetic problems. The mechanism of pathogenesis may be difficult to define in many cases, and extensive research efforts aimed at defining biological mechanisms will be required to capitalize on this new genetic knowledge.

Lymphocyte Antigen Receptor Genes

The basic mechanisms by which T cell antigen receptor (TCR) and B cell immunoglobulin (antibody) genes are rearranged and expressed have been elucidated recently. It is now known that these loci control key steps in all adaptive immune responses. Through complex mechanisms, these genes produce the incredible variety of antibodies and T cell receptors that recognize allergens, autoantigens, and transplantation antigens as well as a very broad spectrum of infectious agents. Without these mechanisms to generate immune diversity we would face almost certain death. Indeed, heroic efforts are required to treat children with inherited deficiencies in the mechanisms that generate diverse antibodies and/or TCR. Some of these children have been treated with new gene therapy strategies with encouraging preliminary results, or treated with bone marrow transplants to replace defective lymphocytes.

Fc Receptors for Immunoglobulin

Immunoglobulin receptors bind antigen:antibody complexes through the Fc portion of the immunoglobulin molecule, and the Fc receptors mediate a variety of functions including antigen elimination and effector cell activation. Distinct alleles of several different human Fc receptors have recently been associated with the risk of developing lupus nephritis. It was found that the allele of FcγRIIa, which has an arginine rather than a histidine at position 131, has a reduced affinity for human IgG_2 and is associated with lupus nephritis in African Americans. Furthermore, the allele of FcγRIIIa, which has a phenylalanine rather than a valine at position 176, has a reduced affinity for both human IgG_1 and IgG_3 and is associated with lupus and lupus nephritis in African Americans and European Americans. This association of low-affinity FcγR alleles with lupus suggests that antigen:antibody complexes would be more likely to remain in the circulation and be available to cause the inflammation of lupus nephritis. This hypothesis provides a new level of understanding and opportunities to test new therapeutic strategies for lupus patients and patients with other immune-mediated disorders who have low-binding Fc receptor alleles and differences in effector cell activation.

Primary Immunodeficiency Diseases

Single gene primary immune disorders are now recognized that cause not only susceptibility to infectious agents due to lack of immune function, but also autoimmune diseases due to dysregulation of immune networks (autoimmune polyendocrinopathy, IL-2 receptor alpha chain deficiency) and defects in apoptosis, or programmed cell death (ALPS, autoimmune lymphoproliferative syndrome, due to Fas defects). Human immunodeficiencies have been critical in demonstrating the roles played by specific genes and immune pathways in the development of normal immune responses.

During the past 5 years a fundamental shift has taken place in the diagnosis, treatment, and genetic management of families suffering from these diseases. It is now possible to understand the specific cause, down to the level of a particular nucleotide misspelling in the genetic code and/or consequent malfunctioning path-

way, for more than 75 distinct forms of immunodeficiency, the majority of heritable human immunodeficiency phenotypes, and particularly the most severe. There are now 19 distinctly recognized forms of combined T and B cell immunodeficiency, for which 17 genes have been identified; 3 forms of agammaglobulinemia, all with identified genes; 10 immunoglobulin subclass deficiencies, 8 with identified genes; 16 forms of well-defined immunodeficient syndromes, with 13 identified genes; 12 neutrophil defects, with 8 identified genes; and 27 complement defects, with 26 identified genes (also see Chapter 3).

New therapeutic strategies are currently available or being pursued now that disease-causing genes are known. For example, neonatal bone marrow transplantation following prenatal diagnosis of severe combined immunodeficiency (SCID) confers earlier engraftment with fewer complications than occur in similarly affected infants diagnosed and treated later in life. Successful *in utero* bone marrow transplantation for SCID has been carried out following prenatal diagnosis. The SCID caused by deficiency of adenosine deaminase was the first disease for which human gene therapy was attempted. Increasingly promising developments in the field of gene transfer may allow correction of defects in maturing lineages of immune cells and hematopoietic stem cells.

New Tools for Genetic Analysis

There are a number of recent technological advances and rapidly growing databases that now allow the study of previously inaccessible human genetic problems or that can dramatically accelerate progress in areas long studied by more laborious methods. Noteworthy among these new tools are those that have identified human gene sequences at an extraordinary rate as part of the Human Genome Project.

Gene Targeting in Mice

Enormous progress has occurred in producing gene-targeted mouse strains for experimental study. Knockout mice in which a particular gene has been inactivated, transgenic mice in which a particular gene has been added or a specific mutation introduced, recombinant inbred strains, and congenic mice provide valuable strategies to identify genes responsible for observed clinical phenotypes. Even newer techniques are beginning to allow mouse genes to be targeted and manipulated in a cell type-specific and/or inducible manner. Figure 15-2 illustrates Cre-loxP-mediated gene targeting in mice as an example. The organization of the mouse genome is approximately 95 percent homologous with the human genome. When a human gene is shown to contribute to disease phenotype, then targeted polymorphisms in the homologous mouse gene can be studied for their contribution to the disease in an appropriate mouse model. This is a very powerful approach to the understanding of human disease and the identification of suitable therapeutic approaches.

The Human Genome Project

The Human Genome Project has already provided a comprehensive map of the human genome, clearly an important foundation upon which much progress will be built. The DNA sequences now available for the human genome are being

exploited by virtually every investigator attempting to understand immunologic disorders and normal physiology. The power of these informatic resources to contribute to the solution of important disease problems will dramatically improve as the DNA sequence is completed over the next few years. Collaborations among clinicians, immunologists, and geneticists are vital to the success of efforts in this area. Excellent examples include recent progress in ataxia telangiectasia and the recent description of ALPS.

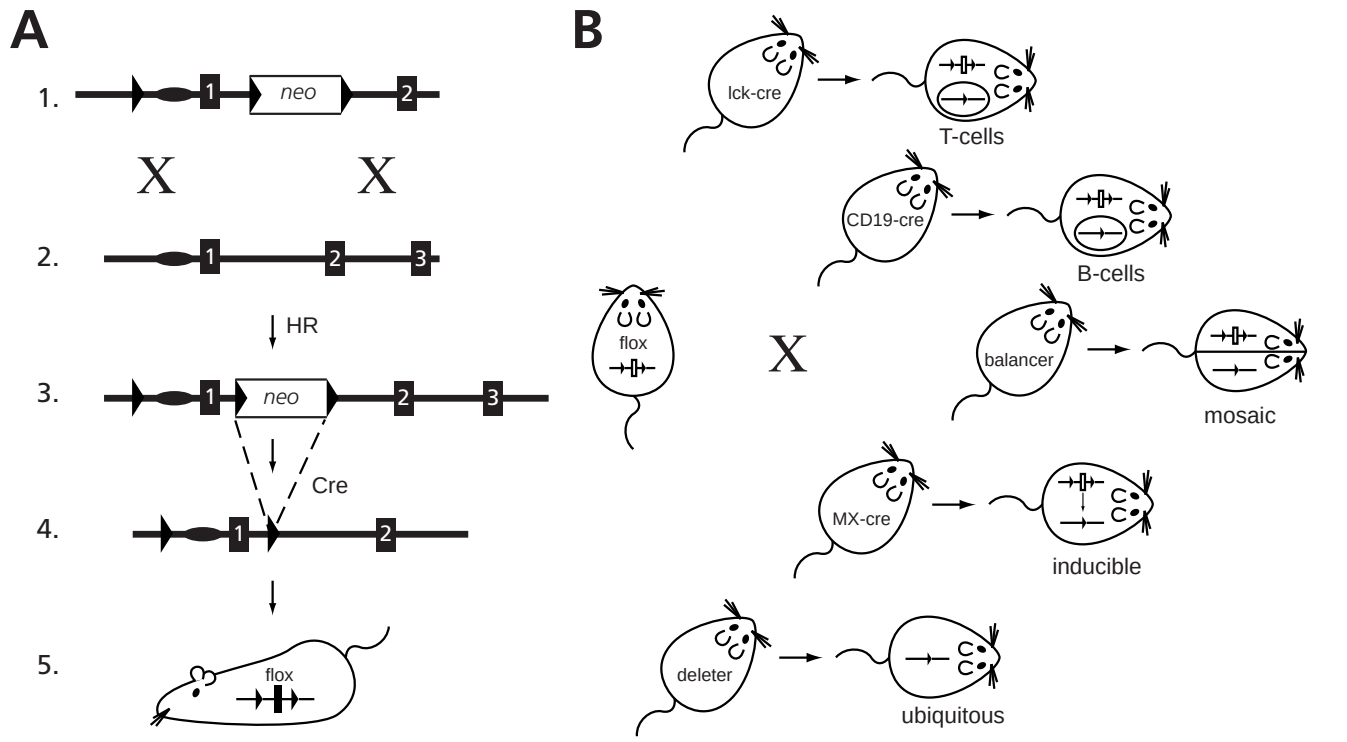


Figure 15-2. The Cre-loxP system for targeting particular genes.

A. The steps used to generate mice that have loxP-flanked target genes (flox). 1. A gene-targeting vector is constructed containing three loxP sites (filled triangles), two of which flank a neomycin resistance (*neo*) gene. 2. Target gene as found in mouse ES (embryonic stem) cells. 3. Some daughter ES cells are resistant to neomycin after homologous recombination (HR). 4. The action of Cre deletes (inactivates) the neomycin resistance gene. 5. Now the loxP sites flank the gene segment.

B. The flox mouse is crossed with five different mice that express Cre recombinase in different ways. The gene segment between the loxP is deleted according to the expression of Cre. The target gene is deleted only in cells expressing Cre but remains active elsewhere.

Source: Rajewsky, K.; et al. Conditional gene targeting. *J. Clin. Invest.* 98:600–603, 1996. Copyright 1996. Reproduced from *The Journal of Clinical Investigation*, 1996, Vol. 98, pp. 600–603 by copyright permission of The American Society for Clinical Investigation.

Analysis of Complex Diseases

A major advance in understanding how to approach complex diseases is under way. The ability to estimate the power of a particular collection of families to provide insight into a genetic disease has recently been substantially improved. Nonparametric methods of analysis, including improvements on the traditional sib-pair methods, are now available, and they have already provided insights into insulin-dependent diabetes (Type 1), inflammatory bowel disease, celiac disease, asthma, and multiple sclerosis, among others. For example, the Type 1 diabetes whole genome scan for disease-linked chromosome regions showed that the MHC is the major locus and that several other loci are involved, thus providing direct information on the inheritance of this complex disease. These approaches will be applied to a host of additional immunologic disease problems in the near future. Disequilibrium methods are useful to localize genetic effects identified first by genome scanning for linkage. However, traditional linkage analysis depends on its ability to fine map disease genes, because in complex disease, having the disease genotype does not ensure that the disease will occur. Linkage disequilibrium and allelic association are attractive approaches, but they will only be successful if large numbers of families (usually thousands from outbred populations) or well-constructed, large case-control studies are available. Moreover, the allelic association approach will rely heavily on the emerging gene map from the Human Genome Project, so that disequilibrium mapping experiments can be directed toward positional and functional candidate genes in regions of linkage. These and other advances will help solve many previously intractable problems in infectious, inflammatory, and immunologic diseases, and results from these studies should allow future development of effective treatment protocols.

Specific Diseases: Problems and Prospects

Diabetes

The enormous investment in understanding Type 1 diabetes led to the first genomic scan of a complex disease. Now we know that many different chromosome regions appear to be related to the risk of developing diabetes; perhaps as many as 20 regions are involved. The HLA antigens and the molecular apparatus that regulates the immune response were already known to be important (Figure 15-3). The coming era will concentrate on defining the functions of newly identified genes and understanding the biological mechanisms that lead to disease. No doubt there will be therapies whose success will vary depending on the genotype, and it will be important to establish the key gene-phenotype-environment interactions that underlie the dramatic differences in disease incidence seen in different countries.

Multiple Sclerosis

Multiple sclerosis (MS), which is long known to have a weak association with HLA-DR2, has been linked to the genetic region in the MHC locus on chromosome 6p21; other suspected candidate genetic regions include the 7q35 T cell receptor beta chain and 14q32 immunoglobulin H chain regions. DR2-bearing extended haplotypes were associated with MS in different patient populations, but the studies do not distinguish between a true

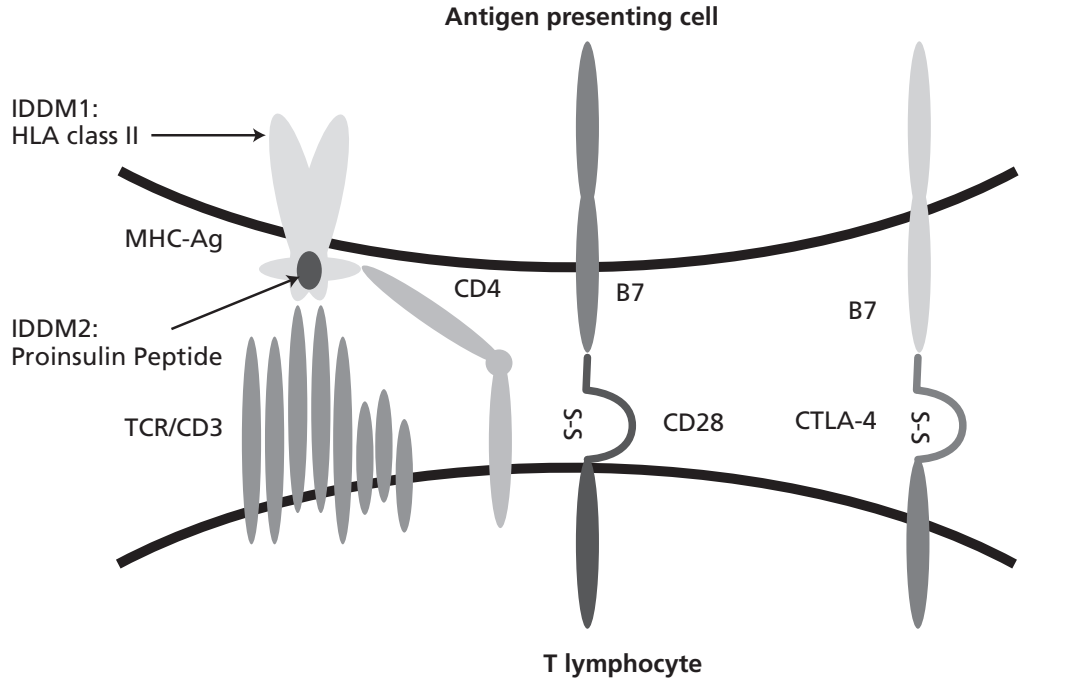


Figure 15-3. Some of the known molecular apparatus that governs the immune response.

The HLA antigens, which are associated with many diseases (Figure 15-1), such as Type 1 diabetes, bind peptides, such as proinsulin peptides. The IDDM2 locus may control the level of proinsulin expression in the thymus, thus affecting self-tolerance to proinsulin, an established autoantigen in Type 1 diabetes. Together, the HLA and peptide are bound by the T cell receptor complex (TCR/CD3). Many other molecules help decide whether this peptide will become the target for the immune response and, if so, what particular kind of immune response will follow. If the genes regulating the extent and magnitude of the immune response to self proteins such as proinsulin are defective, then this initial recognition of peptide by T cells could lead to tissue damage and autoimmunity.

Source: John A. Todd, Ph.D., The Wellcome Trust Centre for Human Genetics, Oxford, England, 1997.

genetic effect and a biologically irrelevant marker of population admixture. Recent whole genome screens of multiple affected member MS families support linkage to the MHC region as well as to additional susceptibility genes. Molecular approaches to find these genes require the availability of large populations of well-characterized affected families, access to a pool of highly polymorphic markers, and multifaceted statistical approaches to data analysis.

Inflammatory Bowel Disease

The first genetic linkages for inflammatory bowel disease were reported recently. Studies of this important disease and many others associated with a genetic

component are usually many years behind the sophisticated analysis achieved thus far for Type 1 diabetes. Major commitments of talent and resources are needed to spur more rapid progress, and appropriate strategies are now available for productive investigation.

Rheumatoid Arthritis

International efforts are under way to collect the pedigree materials, perform the genotyping, and analyze the data from families with multiple cases of rheumatoid arthritis. This disorder is so common and the individual genetic contributions so small that important new information is likely to be produced only by a major effort over an extended period.

Systemic Lupus Erythematosus

Worldwide, more than 700 multicase pedigrees have been collected for the effort to identify genes that confer susceptibility to lupus. Much interest has focused on genes on chromosome 1. Other loci that are likely to be important include at least one HLA gene. As the number of pedigrees increases and the genotyping is completed, the true genetic complexity of lupus should become apparent. Identifying the genes should aid in evaluating prognosis and may lead to unexpected therapies that are likely to be improvements over the nonspecific and toxic therapies now used.

AIDS, Malaria, and Chemokine Receptors

Recently, two human genes were identified that are associated with susceptibility to AIDS and malaria. Interestingly, both genes code for related cellular receptors that normally bind proinflammatory signaling molecules known as chemokines, and both receptors are exploited by the respective microbes as docking sites that enable entry into target cells. Individuals who are genetically deficient in each of these receptors have been identified. Remarkably, the only known consequence of receptor deficiency is resistance to malaria or HIV-1 infection, suggesting that these receptors could be targeted for therapy or prevention without inducing detrimental side effects. Certainly, these discoveries provide a new approach to identify genetic factors underlying other infectious diseases and to develop new therapies for established HIV-1 and malaria infections.

Asthma and Allergic Diseases

Asthma and allergies are extremely common diseases that are increasing in frequency, morbidity, and mortality and have major health, economic, and social effects. They are complex conditions involving multiple stages and pathways that are influenced by both genetic and environmental factors. The genes involved in these processes are now being studied using complex phenotypes of asthma and allergy, as well as asthma- and allergy-associated phenotypes, such as high IgE levels, specific immune responses, and bronchial hyperreactivity (also see Chapters 6 and 7). Many candidate chromosome areas thought to influence susceptibility have been identified using both the candidate gene approach and the genome-wide scan for linkage. For example, the Collaborative Study of the Genetics of Asthma has identified 11 chromosome regions that show evidence for linkage to asthma in the U.S. population and indicate the relative importance

of asthma genes that may vary between ethnic groups. Other studies have suggested association of susceptibility to certain allergens with chromosome 6p (HLA) and possibly chromosome 14 (TCR); serum IgE levels with chromosomes 5q and 12q; nocturnal asthma with chromosome 5q (β2AR); and bronchial hyperreactivity with chromosome 5q. The emerging picture indicates that responses to environmental agents in asthma and allergy are controlled by various combinations of many gene products. Definitive analyses that can predict disease and indicate appropriate treatments will require intense continued investigation of the underlying genetics and an understanding of these genes in pathogenesis.

Applications to Medical Practice

Practical applications of genetic advances are already occurring in some clinical situations in two important ways. First, genetic risk factors have an influence on disease prognosis, and in many situations help distinguish those patients that are likely to have a more difficult illness from those with milder disease. Second, therapies currently exist or are being developed that are only partially successful in patients with certain genes. The genetic revolution now under way will profoundly affect the clinical management of disease as well as raise new ethical and economic challenges for the future.

Summary

Clearly, we are passing through the threshold to a new era in understanding immunologic diseases that will lead to new strategies to control and prevent human health problems. Much of this progress is possible because new genetic tools are available to identify the human genes involved in causing disease. Further impressive progress is likely, given the commitment of the scientific community and the interest of the populace as reflected in the priorities of government leaders. Only when our most talented scientists are able to command the resources needed is this kind of progress possible.

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Research Opportunities

- Identify genes that contribute to disease susceptibility and disease manifestations, as well as the biochemical basis for their functions; understand the pathogenic roles for genes identified by new genetic methods
- Investigate the molecular mechanisms by which genetic variants known to be associated with disease influence disease susceptibility
- Develop and apply methods to identify genes responsible for observed linkages in disorders with complex genetics
- Use genetic interactions to identify phenotypes with particular prognostic risks or with improved therapeutic responses
- Utilize gene-targeting strategies in mice to develop clinically relevant models of human disease for study of mechanisms and potential therapies
- Accelerate gene identification by combining pedigree materials and analyses from multiple research groups to increase the power of the results obtained

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Afterword: Contributions of Basic Immunology Research to Human Health

The past several decades have witnessed far-reaching advances in our understanding of the immune system, allowing more precise manipulation of its components than previously possible. As outlined in this report, these advances are mirrored in myriad newly emerging therapeutic and diagnostic applications. These include novel vaccine strategies, advancements in understanding of both acquired and congenital immunodeficiency, and the transfer of technologies that exploit cells or molecules of the immune system in a wide variety of applications. For example, vaccines against *Haemophilus influenzae* type b, a bacterium responsible for ear infections and meningitis in children, have been developed recently and are in widespread use, limiting the impact of these diseases in group day care and educational settings. This timely advance in vaccination directly reflects an increased understanding of the mechanisms by which the immune system can be induced to respond to bacterial carbohydrates.

Novel vaccine strategies are now under scrutiny, and may afford highly targeted or more effective immunization for a variety of diseases. Molecular genetic investigations in animal models of human disease, as well as similar studies utilizing human tissues, have afforded the direct identification of lesions responsible for heritable immune deficiency syndromes, such as the dysfunctional enzyme in Bruton's agammaglobulinemia. Once further refined, these findings will afford the hope of curative therapies. The application of immunologic tools such as monoclonal antibodies continues to expand, and ranges from new generations of clinical tools to imaginative end-user-based diagnostic tests. Enhanced methods to generate these reagents *in vitro* through molecular manipulations are presently being explored. Furthermore, new methods to emulate the *in vivo* processes involved in generating high-affinity antibodies are under development. These efforts will likely culminate in the ability to engineer and rapidly generate antibodies of high selectivity.

Reflecting on the history of such advances provides insight into how research evolves from probing the most basic questions to eventual everyday utility. It is beyond the scope of this report to trace each example from its inception in the laboratory to its application in human health and disease. Indeed, the clear examples of successful vaccines and the promise of molecular genetic and monoclonal antibody technologies have been detailed at length elsewhere. Here we focus on one example, in which recently gained knowledge of the mechanisms responsible for graft-related disease, coupled with the identification and characterization of molecules that control blood cell production, has provided the means to use bone marrow transplantation in conjunction with hematopoietic growth factors in the treatment of otherwise terminal malignancies such as breast cancer and aggressive lymphomas.

Cancer and Bone Marrow Transplantation

Most blood cells originate from pluripotent stem cells in the bone marrow, which give rise to a wide array of lineage-restricted progenitor cells (see Chapter 2). Since most blood cells have a relatively short lifespan of weeks to months, maintaining their steady-state numbers relies on the continuous production of new cells from stem cells in the bone marrow. Cessation of this production can yield life-threatening shortages of cells, especially erythrocytes, which transport oxygen to tissues, and neutrophils, which help destroy pathogenic bacteria. Most cancer therapies employ chemicals and/or radiation to eliminate malignant tumor cells, but these agents also kill other cell types, including bone marrow stem cells. Although providing an outside source of stem cells after treatment could clearly resolve the difficulty, this strategy was originally confounded by two additional problems. First, the transfer of bone marrow cells between unrelated individuals (allogeneic transfer) starts a complex series of events that culminate in immune rejection of the engrafted stem cells (graft rejection) and/or the induction of graft-*versus*-host disease (GVHD), a life-threatening illness mediated by the donor T cells. Although the magnitude of these problems can be reduced by the transfer of cells between genetically related individuals (semi-allogeneic transfer), the added benefits of this strategy are limited and appropriate donors are not always available. Furthermore, because it normally requires at least 3 to 4 weeks to generate sufficient numbers of newly formed blood cells from the donor bone marrow cells, the patient is at risk for a significant period before realizing the benefits of the stem cell transfer. In the past several decades, two diverse areas of immunologic research have culminated in the development of clinical strategies that vastly improve the success of bone marrow transplant and reestablishment of hematopoiesis.

T Lymphocytes and Graft-versus-Host Disease

T lymphocytes regulate the activity of other lymphocytes and eliminate virus-infected cells, while myeloid-lineage cells such as macrophages and neutrophils destroy bacteria and other potential pathogens. Each of these cells is found in the blood supply, the lymphatic organs, and the bone marrow. Since many cancers and their related therapies lead to immunodeficiency, successful allogeneic marrow transplants are usually thwarted by graft-*versus*-host disease rather than by graft rejection. The basis for GVHD, including the cells responsible and the nature of the immunologic reaction, was poorly understood as late as the early 1970s. Several avenues of research allowed further insight: experiments showing that GVHD is a result of immune system function, the description and characterization of the T cell receptor for antigen, the identification of T cells as responsible for GVHD, and an understanding of how T lymphocytes are selected during their development.

While seemingly straightforward, the rationale for these experiments was directly linked to an increasingly sophisticated knowledge of how T lymphocytes bind antigens, as well as the mechanisms that allow T cells to distinguish self from nonself elements. The development of

these conceptual frameworks was spearheaded by intense research efforts defining the requisites for T cell antigen recognition and their selection during thymic development, as well as by programs aimed at defining and characterizing the T cell receptor for antigen. With these emerging insights, genetically defined mouse strains were used to demonstrate that removal of mature T lymphocytes from marrow donor cells prevented GVHD, thus showing that mature T lymphocytes were the primary cell type responsible for induction of GVHD. This observation led to clinical strategies that remove most mature T lymphocytes from human marrow donor cells, thus ameliorating many GVHD-associated problems and increasing the probability of successful allogeneic marrow transplantation. Despite this advance, additional obstacles prevented the adoption of semi-allogeneic marrow transplantation as a means of cancer therapy. In particular, it was necessary to shorten the time interval between stem cell transfer and generation of adequate numbers of donor-derived blood cells.

Factors Controlling Hematopoiesis

The control of erythrocyte and neutrophil production is a complex process whose details remain obscure. Initial advances in this area relied on the development of *in vitro* colony assays in which stem cells and/or their recent progeny form distinct colonies in semisolid media. This system allowed the identification of progenitor cells for each type of blood cell *via* its unique morphology, and permitted the introduction of soluble factors that can effect the growth and/or differentiation of stem cells and their progeny.

Supplementing this assay with a host of soluble factors led to the notion that the development of erythrocytes and neutrophils is controlled by distinct soluble molecules that augment the growth and differentiation of their respective progenitor cells. This conclusion was drawn from experiments showing that erythropoietin increased the number and size of erythroid colonies but had no effect on other cell types. Subsequent molecular characterization of this hormone and its receptor has confirmed this notion, identifying erythropoietin as a key soluble mediator of erythropoiesis. Analogous studies of the growth and differentiation among myeloid lineage cells identified a soluble factor in the marrow and blood that specifically increased myelopoiesis. This protein, termed granulocyte-macrophage colony-stimulating factor (GM-CSF), was subsequently shown to increase both proliferation and function of most myeloid lineage cells, including macrophages and neutrophils. This information, together with knowledge of erythropoietin and its ability to increase erythrocyte production, opened the possibility of using these mediators of normal hematopoiesis in clinical settings.

Treatment of Marrow Transfer Recipients With Hematopoietic Factors

As discussed above, the survival of cancer patients who require allogeneic or autologous stem cell transfer is often threatened by the loss of erythrocytes and neutrophils before donor-derived stem cells can sufficiently repopulate the recipient. Clearly, the ability to hasten this process represents an unequivocal advantage for these patients. Recent studies showing accelerated neutrophil recovery in lym-

phoma patients undergoing allogeneic marrow transplantation support the use of GM-CSF to increase the patients' ability to thwart potentially life-threatening infections. This strategy has also proven useful for lymphoma and breast cancer patients undergoing autologous stem cell transfer. Furthermore, erythropoietin, sometimes used with GM-CSF, has allowed increased erythrocyte production in patients undergoing either autologous or allogeneic transfer. Together, these studies support the use of GM-CSF and erythropoietin as part of a standard treatment regimen for breast cancer or lymphoma patients requiring allogeneic or autologous marrow transfer. These advances have clearly led to enhanced survival and reduced morbidity and mortality in a variety of clinical settings. For example, hospital stays of patients undergoing therapy for breast cancer were reduced by as much as 17 to 20 percent. Similarly, a number of clinical trials have shown that the need for medical intervention or the longevity of antibiotic treatment can be reduced. In addition to the obvious improvement in therapeutic success and quality of life afforded by these approaches, several studies suggest that they have yielded significant savings in health care costs. It has been estimated that growth factor therapy can reduce health care delivery costs by half in certain transplantation scenarios, primarily through reductions in hospitalization time or reduced costs during hospitalization.

This one example of the benefits of basic research highlights the need for continued intense efforts to understand the immune system within the physiological context of the whole human organism. It is also clear that information obtained from multiple avenues of investigation, ranging from molecular analyses of far distant species to clinical studies of human disease, contributes critical clues for ultimate breakthroughs in the translation of basic research to therapeutic applications.

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Glossary

Activation-induced cell death (AICD) – lymphocyte death by apoptosis that occurs following an immune response

Adaptive immunity – antigen-specific responses of T and B lymphocytes that are induced after recognition of antigen; also called acquired or inducible immunity

Adhesion molecules – cell surface proteins that mediate intercellular adhesion or binding of cells to extracellular matrix components; adhesion molecules play important roles in leukocyte migration and T cell interactions with antigen-presenting cells

Adjuvant – a compound or mixture of compounds that nonspecifically enhances the immune response to an antigen

Affinity – the binding strength between two molecules; affinity is generally expressed as an equilibrium constant for association or dissociation

Affinity maturation – increase in average affinity over time of the population of antibodies produced in an immune response

AIDS (acquired immunodeficiency syndrome) – disease of the immune system that results from infection with HIV (human immunodeficiency virus) and is characterized by severe T cell depletion and high susceptibility to opportunistic infections

Allele – one or more alternative DNA sequences for a gene at a particular chromosomal locus

Allergen – a noninfectious antigen that causes hypersensitivity or an allergic response

Allergic reaction – response of preexisting antibodies or memory T cells to an allergen

Allergic rhinitis – allergic response in the nasal tissues causing sneezing and runny nose

Allergy – specific antibody- or T cell-mediated immune reaction to a previously encountered allergen, usually denoting an immediate hypersensitivity reaction

Allogeneic – tissues, organs, or individuals of the same species that are antigenically different due to genetic differences

Allograft – tissue or organ transplanted between allogeneic individuals

Alloreactivity – immune response to allogeneic cells

Anaphylaxis – an antigen-specific allergic response that causes vasodilation, airway swelling, possible suffocation and death

Anergy – a state of unresponsiveness caused by functional inactivation of T or B cells without cell death

Angiogenesis – the development of new blood vessels

Antibody – a soluble protein of the immunoglobulin class that is produced by B cells in response to infection or immunization; an antibody binds specifically to the antigen that induced its production to neutralize the antigen or make it more susceptible to destruction by phagocytic cells

Antigen – a molecule that binds to specific lymphocyte antigen receptors

Antigen presentation – the display of antigen fragments bound to MHC molecules on the surface of antigen-presenting cells; the process by which antigens are presented to T cells for specific recognition

Antigen-presenting cells – specialized cells in the body, such as macrophages, dendritic cells, and B cells, that can produce antigen-MHC displays for the stimulation of T cells, or follicular dendritic cells that present antigen-antibody complexes for the stimulation of B cells

Antigen processing – the degradation of protein antigens by a variety of pathways within antigen-presenting cells to form peptide fragments suitable for binding to MHC molecules and presentation to T cells

Antigenic peptide – the fragment of a protein antigen that is generated by enzymes within antigen-presenting cells and binds to a MHC class I or class II molecule for recognition by T cells

APC – antigen-presenting cell

Apoptosis – process of cell death regulated by intracellular mechanisms that cause nuclear degeneration and other characteristic cellular changes; apoptotic cells are phagocytosed by tissue macrophages to avoid damage to surrounding cells; apoptosis is also called programmed cell death

Arachidonic acid – a lipid constituent of cell membranes; several metabolites of arachidonic acid are inflammatory agents

Asthma – a disease of the lung that causes airway obstruction, inflammation, and hypersensitivity to multiple stimuli

Atopy – a genetically determined hypersensitivity condition that includes reactions such as asthma, eczema, and rhinitis

Autoantibody – a specific antibody that binds to a self antigen

Autoimmune disease – a T or B cell-mediated disease caused by specific immune responses to self antigens

Autologous – product or component of an individual

B cell – a small white blood cell, also called B lymphocyte, that expresses a surface immunoglobulin antigen receptor and can be stimulated to secrete the receptor in a soluble form as an antibody

B cell antigen receptor (BCR) – immunoglobulin molecule expressed on B cell surface that binds specific antigen; the BCR on each B cell has a unique sequence in the VDJ region and therefore a unique antigen specificity

B7 molecules – major costimulatory proteins expressed by antigen-presenting cells and required in many T cell:antigen-presenting cell interactions for optimal T cell stimulation; two forms are known: B7-1 and B7-2

Bacterium – one of a wide variety of single cell microscopic organisms

BAL (bronchial-alveolar lavage) – irrigation, or washing out, of the lung

Basophil – a white blood cell that contains numerous secretory vesicles that release histamine and other soluble mediators in certain immune responses

Bcl – family of intracellular proteins containing members that promote apoptosis and those that inhibit apoptosis

Bone marrow – the soft material that fills bone cavities and contains hematopoietic stem cells, maturing red and white blood cells that derive from these stem cells, and some mature lymphocytes and cells of other lineages; bone marrow is the site of B cell maturation in mammals

Caspase – a family of protease enzymes that mediate cell death

CD – designation for some surface marker proteins (cluster of differentiation proteins), such as CD3, CD4, CD8, CD28, and CD40, found on lymphocytes and other cells, that play individual roles in cellular activation and regulation

CD28 – T cell surface receptor that binds B7 molecules to deliver costimulatory signal to T cells

CD3 complex – nonpolymorphic, related set of proteins that associate with T cell antigen receptors and mediate signals to T cells after antigen binding; three types of CD3 proteins exist: γ, δ, and ε; a nonpolymorphic ζ dimeric protein is also found in the complex of antigen receptor with CD3

CD40 – protein expressed on B cells and other cell types that transmits signals into the cell to regulate responsiveness; CD40 is activated by binding CD40 ligand (CD40L), which is induced on activated T cells

Cell cycle – the four phases of the process of cell division: G1, S, G2, and M

Cell-mediated immunity – see cellular immunity

Cellular immunity – immune protection provided directly by immune cells such as cytotoxic T cells

Centroblast – large, proliferating B cell found in germinal centers of lymphoid organs; somatic mutation of immunoglobulin is thought to occur at this stage of mature B cell differentiation

Centrocyte – small resting B cell that follows the centroblast stage; its fate is determined by interaction with antigen, and it may become an antibody-secreting cell or a memory cell, or it may die

Chemokines – soluble proteins that mediate cell migration and activation during immune and inflammatory responses

Chemotaxis – directed cellular movement in response to gradient concentrations

of a variety of cell-attracting, or chemoattractant, factors

Chromosomes – DNA structures in the nucleus that contain the genes

Complement – a complex set of blood proteins that are activated in cascade fashion to form structures that can lyse cell membranes; certain complement components bind antibody-antigen complexes and enhance immunity through complement receptors on immune cell surfaces

Constant region – the relatively invariant portion of an immunoglobulin or TCR receptor protein

Coreceptor – denotes a nonpolymorphic protein on a lymphocyte surface that increases the ability of the antigen receptor to bind antigen; coreceptor usually refers to the CD4 or CD8 molecule on T cells and the CD19/CD21/TAPA complex on B cells

Corticosteroids – generally immunosuppressive and anti-inflammatory steroid products of the adrenal cortex

Costimulatory signal – second signal often needed to activate lymphocytes in addition to the antigen-specific signal

Crossreaction – response to an antigen different from, but structurally related to, the known antigen recognized by a lymphocyte

CTL – cytotoxic T lymphocyte

CTLA-4 – B7-binding protein induced on the surface of T cells during activation; it is thought to mediate downregulation of T cell responses

Cyclosporin A – a potent T cell-suppressive drug derived from fungi and very useful for the prevention of transplanted allograft rejection

Cytokine – soluble protein produced by one or more cell types that binds to its specific cytokine receptor to influence the course of an immune response

Cytotoxic T lymphocytes – a subset of T cells that usually carries the CD8 marker and is efficient at killing virally infected cells and tumor cells based on recognition of intracellularly derived antigens complexed with MHC class I molecules on the target cell surface

Degranulation – release of granules, or vesicles, containing soluble mediators from mast cells or basophils into the extracellular milieu

Delayed-type hypersensitivity (DTH) – inflammatory memory CD4 T cell response that occurs within hours or days after antigen exposure in the skin

Dendritic cells – white blood cells also found in T cell areas of the spleen, lymph node, and other lymphoid organs where they are characterized by long cellular processes, or dendrites, and potent antigen-presenting activity for T cells

DNA (deoxyribonucleic acid) – nucleic acid found in the nucleus that constitutes the genetic material of the cell

Dominant negative transgene – mutant form of a gene introduced into a genome to encode a protein that

inhibits the function of the normal protein

Drosophila – fruit fly; used in genetic studies

Ectopic – located in other than the normal position

Edema – tissue swelling that results from injury or disease

Effector T cells – antigen-activated T cells armed to secrete cytokines or kill antigen-positive cells

Eicosanoids – family of arachidonic acid metabolites that can regulate immune and inflammatory responses

Endothelium – the epithelial cell layer that lines the heart and the blood and lymph vessels

Enzyme – a protein that catalyzes chemical changes in other molecules

Eosinophil – a white blood cell that can secrete potent antiparasitic factors and enzymes that contribute to inflammatory responses; often present in abnormally high concentrations in allergic individuals

Eotaxin – cytokine produced by Th2 cells that functions in eosinophil recruitment and activation

Epitope – the region on an antigen that binds to a T or B cell antigen receptor

Epstein-Barr virus (EBV) – a herpes virus that infects human B cells

Erythroid – pertaining to the red blood cells (erythrocytes)

Etiology – the study of factors that initiate disease

Experimental allergic encephalomyelitis (EAE) – animal disease model of multiple sclerosis in which T cells reactive with myelin proteins are induced by immunization resulting in central nervous system inflammation

Extravasation – movement of cells from the blood into a tissue

Fas – surface protein that mediates signals for apoptosis in many cell types, including lymphocytes; Fas ligand (FasL) is induced on activated T cells and engages Fas to initiate cell death

Fc – the portion of an immunoglobulin/antibody molecule that is derived from the constant region and can bind specific cellular receptors and certain complement factors

Fc receptors – antibody isotype-specific cellular receptors that bind the Fc portion of antibody molecules and play important roles in antigen clearance or mast cell/basophil degranulation

Follicular dendritic cell – specialized cell found in germinal centers and characterized by its very long cellular processes that tightly bind antibody-antigen complexes for long periods of time; this cell is thought to be important in maintaining an antigen depot to keep memory B cells in the body

Fungi – eukaryotic single-celled organisms such as yeasts and molds

Gamete – a reproductive cell; ovum or spermatozoon in humans

Gene – a discrete segment of DNA that encodes a functional protein or RNA sequence

Gene knockout mice – mice in which a specific gene has been inactivated by homologous recombination

Gene therapy – the introduction of a normal gene into cells to replace the function of a defective gene

Genetic association – correlation of a particular gene sequence with a functional activity or disease state

Genetic loci – chromosomal regions

Genome – the complete set of genes in an organism

Germinal centers – microenvironments in the primary follicles of secondary lymphoid tissues such as the spleen or lymph nodes; locations where B cell activation, proliferation, and somatic mutation occur

Glycoproteins – proteins containing covalently bound carbohydrate that are frequently found on cell surfaces

Granulocyte – a white blood cell that contains granules, or vesicles, filled with soluble mediators; neutrophils, eosinophils, and basophils are granulocytes

Granulocyte-macrophage colony-stimulating factor (GM-CSF) – cytokine important for the growth and differentiation of monocytes, macrophages, dendritic cells, and granulocytes

Granzymes – apoptosis-promoting enzymes produced by cytotoxic T and NK cells and stored in intracellular granules

GVHD (graft-*versus*-host disease) – disease caused by attack of donor T cells on recipient cells of a different genotype; frequent and potentially fatal consequence of allogeneic bone marrow transplantation

Helminth – a parasitic worm

Hematopoiesis – the process by which blood cells are formed

Hematopoietic stem cell (HSC) – the pluripotent precursor cell from which all blood cells are generated

Heterozygous – having two different alleles of a gene, one on each chromosome

Histamine – a vasoactive compound stored in mast cell granules and released when IgE-antigen complexes trigger degranulation; causes the symptoms of smooth muscle contraction and local blood vessel dilation in allergy

Histocompatibility – the ability to accept a graft from a genetically different individual

HIV (human immunodeficiency virus) – a retrovirus that depletes CD4 T cells resulting in immunodeficiency and AIDS

HLA (human leukocyte antigen) – designation for the human major histocompatibility gene locus and proteins

Homeostasis – physiological equilibrium

Homologous – similar in structure or function

Humanized antibody – antibody of animal origin that has been genetically engineered to replace conserved sequences with homologous regions of human antibody in order to preserve human effector functions and minimize human anti-animal immune responses that can destroy the antibody

Humoral immunity – specific immune response mediated by antibodies

Idiopathic – refers to a disease of unknown origin

Idiotype – unique region on an antibody or TCR that corresponds to the antigen-binding region

Immediate hypersensitivity – antibody-mediated response that occurs within minutes of antigen exposure in sensitized individuals

Immune complex – aggregate of antigen bound to antibody

Immune deviation – dominance by one type of immune response; often used to refer to the preferential activation of Th1 or Th2 cells

Immune response – the individual or aggregate reactions of the immune system to foreign substances

Immune system – the specialized organs, cells, and molecules responsible for innate and adaptive immunity

Immunization – deliberate induction of an adaptive immune response to generate immune memory

Immunogenetics – the study of genes involved in immune system development and immune responses

Immunogenic – able to induce an immune response

Immunoglobulin (Ig) – the family of structurally related proteins that individually contain both highly variable antigen-binding regions and conserved constant regions; membrane-bound immunoglobulin serves as the B cell antigen receptor, and the secreted form is the antibody

Immunologically privileged sites – anatomical locations such as the brain and eye that do not elicit strong reactions to allografts because they are local sites of potent immunosuppressive molecules

Immunosuppression – inhibition of immune responses; often used to designate the nonspecific inhibition of the entire immune system

In vitro – laboratory studies conducted with tissues, cells, or molecules isolated from intact organisms

In vivo – studies performed within intact organisms

Inflammatory response – local reaction to tissue injury or infection characterized by the infiltration and accumulation of certain white blood cells, fluids, and proteins

Innate immune system – cells and soluble factors that are present constitutively to respond to classes of microorganisms in a non-antigen-specific manner

Insulin-dependent diabetes mellitis (type 1 diabetes) – disease caused by autoreactive T cell destruction of the pancreatic β cells that produce insulin

Integrin – a family of surface proteins that mediate cellular adhesion

Intercellular – direct or indirect interactions between cells

Interferon (IFN) – class of cytokines produced to fight viral infection, mediate inflammatory reactions, or modulate specific antibody or T cell responses; major interferons include IFNα, which is produced by leukocytes, IFNβ, produced by fibroblasts, and IFNγ, produced by T and NK cells

Interleukin (IL) – class of cytokines involved in signaling between leukocytes and other cells; name used for some cytokines, such as IL-2, IL-3, IL-4, IL-5, etc.

Intracellular – within a cell

Isotype – subclass of immunoglobulin distinguished by its constant region sequence; isotypes include IgM, IgD, IgG, IgA, and IgE

Isotype switch – switch in B cells from IgM synthesis to production of different isotypes that still have the same antigen-binding region

Killer T cell – see cytotoxic T lymphocyte

Knockout animal – see gene knockout mice

Langerhans' cell – epidermal dendritic cell that processes foreign antigen in the skin and then migrates to a lymph node for presentation to T cells

Latency – microbial infection of a cell or tissue that does not result in replication or cellular damage until the microbe is reactivated to multiply

Leukocytes – white blood cells: lymphocytes, neutrophils, basophils, eosinophils, and monocytes

Leukotrienes – arachidonic acid metabolites with potent effects on the immune system and other cell types

Ligand – a molecule that binds to another molecule; generally used to refer to the complementary protein, or counter-receptor, that binds to a cell surface receptor

Lipopolysaccharide (LPS) – bacterial component that contains both lipid and carbohydrate; LPS is a potent B cell activator

Lymph nodes – widely distributed lymphoid organs that are linked by lymphatic vessels and are accessible to the circulation; sites of antigen collection and lymphocyte activation

Lymphatic system – a network of lymphoid channels that drains tissue fluids and returns lymphocytes to the blood

Lymphocytes – small white blood cells derived from hematopoietic stem cells that circulate and respond to foreign antigens; T and B lymphocytes express highly diverse antigen receptors that induce antigen-specific immune responses, and NK cells have a collection of monomorphic receptors that

regulate the direct killing of tumor cells and virally infected cells

Lymphomyeloid – refers to the cell lineage that includes both lymphocytes and myeloid cells

Lymphotoxin (LT, TNFβ) – an inflammatory cytokine secreted by Th1-type CD4 cells

Lysis – destruction of a cell

Macrophage – a large migratory cell that can engulf and eliminate pathogens and particles by the process of phagocytosis; T and NK cell products activate macrophages to destroy intracellular pathogens and induce more potent antigen-presenting activity

Mast cell – large granular cell found in the dermis and in submucosal tissues that can be triggered by IgE:allergen complexes and other stimuli to produce immediate allergic responses

Memory – the ability of the adaptive immune system to "recall" prior exposure to an antigen and respond with enhanced kinetics and magnitude; the generation of potent memory T and B cells is the goal of vaccination

Memory cells – B or T cells generated after antigenic stimulation that persist for long periods of time and are available for rapid responses after repeat encounter with the same antigen

MHC (major histocompatibility complex) – a highly polymorphic cluster of genes that encode the MHC class I and class II molecules needed to present antigens to T cells, and that encode other proteins important for antigen presentation and immune activation; MHC class I and MHC class II proteins usually differ between individuals and are the major antigens involved in the rapid rejection of transplanted organs and tissues

Micrometastases – small sites of tumor growth at locations different from the parental tumor

Microorganism/Microbe – organism of microscopic size that can cause disease

Minor histocompatibility antigens – a variety of protein antigens that differ between individuals and stimulate weaker histoincompatible responses than do MHC proteins

Monoclonal – homogeneous antibodies or cells that are produced from a single cell precursor; monoclonal antibodies or T cell clones have a single specificity

Monocyte – the white blood cell precursor of the macrophage

Mucosa – an epithelial, mucous membrane surface lining body channels such as the lung and intestines

Multiple sclerosis – degenerative neurological disease in humans thought to be mediated by autoimmune T cells and inflammatory cells

Multipotent (Pluripotent) – able to develop along several different pathways

Murine – of or relating to mice or rats

Mycobacteria – rod-shaped microorganisms related to the bacillus that causes tuberculosis

Myeloid – cell lineage that includes monocytes, neutrophils, eosinophils, basophils, and megakaryocytes

Naïve lymphocytes – T or B cells that have not yet responded to their antigen

Negative selection – generally refers to the deletion of self-reactive T and B cells in the thymus and bone marrow, respectively

Nephritis – kidney inflammation

Neurohormone – a hormone that activates neuronal cells

Neurotransmitter – a soluble compound produced in the nervous system that transmits signals to neuronal cells

Neutrophil – a large white blood cell that is phagocytic and important in defense against extracellular pathogens

NK (natural killer) cell – a non-T, non-B lymphocyte with the intrinsic ability to recognize and kill certain tumor cells and virally infected cells

Nosocomial – originating in a hospital

Nucleotide – the unit component of DNA and RNA

Oncogene – a gene whose mutation is associated with cancer development

Opsonization – the process by which microbes are made more sensitive to phagocytosis; certain antibodies, complement components, and other proteins can bind to microbes and promote their uptake by phagocytic cells

Organism – an individual living entity

Parasites – organisms such as protozoa and worms that are sustained by living within another organism

Passive immunization – transient immune protection mediated by the injection of specific antibodies or the transplacental transfer of maternal antibodies to the fetus

Pathogen – an infectious organism that causes disease in its host

Pathology – the damage caused to normal tissues by disease

Peptide – an amino acid sequence smaller than a protein; a protein fragment that is composed of at least two amino acids

Perforin – a pore-forming protein produced by cytotoxic T and NK cells that can lyse target cells by creating large holes in the target cell membrane

Peripheral tolerance – lymphocyte tolerance that is acquired at the mature cell stage, outside of the thymus or bone marrow, to inhibit or delete self-reactive, functionally competent T and B cells

Peyer's patches – lymph node-like structures that line the small intestine; important in mucosal immunity

Phagocyte – a cell that can ingest large quantities of particulate inert or living foreign material into vesicles called phagosomes, which can play important roles in the destruction of pathogens

Phagocytosis – the process by which cells ingest foreign materials

Phenotype – the expressed characteristics of an individual that are determined genetically and environmentally

Phosphatase – a class of enzymes that remove phosphate groups from molecules

Plasma cell (plasmacyte) – a fully differentiated B cell stage characterized by antibody secretion

Polymorphism – different forms of the same basic gene or protein

Positive selection – protection of T lymphocytes from default death pathways by recognition of self MHC antigens in the thymus during maturation; B lymphocytes may undergo a similar positive selection process, but the recognition requirements are not yet known

Preclinical – experimental treatment protocols performed in animals to determine their likelihood of success for human therapy

Progenitor – intermediate stage of differentiation between hematopoietic stem cells and mature lineages of immune cell types

Programmed cell death – see apoptosis

Proliferate – reproduce to expand in number

Promoter – the region of a gene that regulates the synthesis of RNA from DNA

Prostaglandins – a family of arachidonic acid metabolites that can influence immune cell responses

Protease – a class of enzyme characterized by the ability to degrade proteins into peptides or amino acids

Protein tyrosine kinase – class of enzymes that catalyze the addition of phosphate groups to tyrosine residues on proteins; these enzymes play critical roles in receptor-mediated cellular activation

Proteins – long sequences of amino acids that constitute one of the major classes of organic compounds in the body; proteins may also contain carbohydrate or lipid

Rag-1 and Rag-2 – enzymes that are required for the rearrangement and assembly of T and B cell antigen receptor genes

Receptor – a cell surface molecule that binds to a specific complementary molecule

Recombination – process by which genetic sequences can be relocated on chromosomes

Repertoire – used to refer to the multitude of antigen receptor specificities found on T and B lymphocytes in the body

Rheumatoid arthritis – inflammatory disease of the joints thought to be caused by autoimmune lymphocytes

Sensitization – induction of an immune response by natural exposure to antigen or deliberate vaccination, that leads to a more vigorous response upon reexposure to the antigen

Sepsis – potentially fatal microbial infection in the blood

Serum – the liquid component of blood that remains after clotting

Severe combined immune deficiency (SCID) – severe primary immunodeficiency disease caused by the absence of T cells or of both T and B cells

SLE (systemic lupus erythematosus, or lupus) – a human autoimmune disease characterized by autoantibodies, including antibodies that react with DNA; antibody-antigen complexes deposit in tissues and cause disease

Somatic hypermutation – the process by which the portions of immunoglobulin genes that encode the antigen-binding regions are mutated in mature B cells during antigen responses

Spleen – lymphoid organ important for immune responses to antigens in the blood

Stem cell – precursor cell from which all cell types of a tissue derive

Stroma – the supporting tissue of an organ

Surrogate light chain – complex of two nonpolymorphic proteins (VpreB and λ5) that binds to immunoglobulin heavy chains to form a pre-B cell receptor critical for B cell maturation

Synergism – the action of two or more molecules or cells that produces more than expected from either agent alone

Syngeneic – genetically identical

T cell – a small white blood cell, or lymphocyte, that matures in the thymus and expresses a specific antigen receptor of the αβ or γδ class; activated T cells produce cytokines, directly interact with B cells to promote antibody production, and mediate direct cytotoxicity of antigen-positive target cells; subsets include CD4 and CD8, as well as Th1 and Th2 types of CD4 cells

TCR – T cell antigen receptor

Teratogenesis – the production of defects in a developing embryo

Th – refers to subsets of CD4 T cells, such as Th1, Th2, and Th3, which are distinguished by the types of cytokines they produce upon activation

Thymocyte – developing T cell found in the thymus

Thymus – a primary lymphoid organ located in the upper chest area; the thymus is the site of T cell maturation

Tolerance – the state of immune unresponsiveness to a specific antigen

Transcription – the process by which RNA is synthesized from DNA

Transcription factor – protein that plays a role in regulating the synthesis of RNA from DNA

Transfection – the artificial introduction of a DNA segment into a cell

Transformation – the process by which normal cells become cancer cells

Transgene – foreign gene inserted permanently into the genome

Transgenic mice – mice in which an inheritable transgene has been inserted for the functional study of that gene

Transplantation – the grafting of an organ or tissue

Trophoblast – the tissue layer that supplies nutrition to a developing embryo

Vaccine – a substance containing specific antigen from an infectious organism that is injected to induce protective immunity without disease; vaccines may consist of whole or attenuated organisms, killed organisms, or isolated antigens combined with adjuvants

Variable region (V region) – the portion of immunoglobulin or T cell receptor genes and proteins that is highly variable in sequence and encodes the contact surface for antigens

Vasoactive – having the ability to constrict or dilate blood vessels

Vector – term for bacterium or virus engineered to include new DNA sequences and used to introduce the new DNA into cells; used in vaccine design to introduce microbial antigen sequences to the immune system

Virus – an infectious microorganism that must replicate in a living cell of a host

White blood cell – see leukocyte

Xenograft – tissue or organ from one species grafted into an individual from another species

Xenotransplantation – grafting of tissues or organs between species